Niche partitioning in similar, closely related sympatric bat species?
A mechanical approach.

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academic degree Doctor of
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submitted by

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1. Summary / Zusammenfassung

SUMMARY

Niche partitioning is an important process that enables different species to coexist in the same areas. The partitioning of niches can prevent out-competition of one species by another species and therefore stabilizes coexistence. Famous examples of niche partitioning among closely related species are the Darwin finches and the cichlid fishes. In many bat species, adaptations for niche partitioning have been found, e.g., clear differences in habitat use, foraging modes, foraging activity patterns, echolocation, size or wing shape can be observed. However, in some closely related and similar species, adaptations for the partitioning of niches are small and therefore hard to find.

In this dissertation, the focus was on two ensembles of closely related, sympatric and similar bat species that occur in Europe. Species from both ensembles are known to forage close to or within dense, structured vegetation. In this habitat, manoeuvrable flight is essential for effective prey pursuit and avoiding collisions with vegetation. The hypothesis is that resource partitioning in the studied species is affected by differences in morphology and, hence, the ability to manoeuvre within vegetation.

Therefore, the external morphology of the species was compared for adaptations to foraging style and for differences among species. This was done by taking wing pictures (standardized pictures of the bats with outstretched wings) of all individuals; these photographs were later analysed in regard to the measures taken and the size-independent ratios of the wing, body and tail. Additionally, each species' ability to fly in artificial vegetation was tested for manoeuvrability performance. The differing densities of vegetation, such as trees and bushes in a forest, were simulated with an obstacle course with varying obstacle spacing. Furthermore, the differences in manoeuvrability performance were related to the morphological measurements.

The first ensemble tested consisted of the greater mouse-eared bat (*Myotis myotis,* Chiroptera, Vespertilionidae) and the lesser mouse-eared bat (*Myotis blythii,* Chiroptera, Vespertilionidae). The two species differed in their overall size, wing-tip shape and tail-to-body length ratio. The lesser mouse-eared bat performed better (fewer obstacle touches or landings) in the obstacle course than the greater mouse-eared bat and was, therefore, considered to be more manoeuvrable. Although differences in the wing-tip shape were observed, the most important characteristic affecting manoeuvrability in both species was found to be the newly defined tail-to-body length ratio. Additionally, when comparing two bats with injured wing membranes with unharmed bats of the same species, no differences in manoeuvrability could be found, even when the wing shape was asymmetric. Therefore, morphometric differences between the two species in their overall size and, more importantly, in their tail-to-body length ratio were postulated to be the main physical characteristics that provide evidence of adaptation to different foraging and feeding strategies.

The second ensemble studied consisted of the five European horseshoe bat species (Chiroptera, Rhinolophidae). The five species differed mainly in their overall size, even though there was overlap among the three intermediate-sized species for some variables (arm-wing area, tail length, tail area, body length and body area). The smaller species performed better than the larger species in the obstacle experiment, with the exception of *Rhinolophus blasii*. Although *R. blasii* is the second smallest bat, its performance was most similar to that of the second largest bat, *R. mehelyi*. The manoeuvrability performance of all five species improved throughout the experiment but not in consecutive trials. When relating the manoeuvrability performance to morphometric measurements, the overall size differences, and especially a shorter body length, seemed to play a major role in better manoeuvrability.

In regard to manoeuvrability, the smallest species, *R. hipposideros,* and the middle-sized species, *R. euryale,* are best adapted to forage within dense vegetation. The species, *R. blasii*, *R. mehelyi* and *R. ferrumequinum,* seem less well adapted to forage in dense vegetation. The results show that these bats are well adapted to different microhabitats within the habitat where they occur sympatrically. However, the differences found among the five species are small, and when compared with other species, show that these five species are all very well adapted to forage within vegetation. In a similar experiment with *Myotis myotis* and *M. blythii* (first ensemble), both species performed more poorly in the obstacle course than any of the five horseshoe bat species.

Finally, different common methods for measuring external morphology (traditional morphometrics) were closely studied and tested for their ability to discriminate among species. In addition to the traditional morphometrics, a newer method, rarely used until now (in regard to external morphology), was included: geometric morphometrics. Geometric morphometrics are increasingly used in the biological sciences to analyse variation in shape and to discriminate among species and populations. Here, the ability of traditional versus geometric morphometric methods was compared for discriminating among European horseshoe bats based on morphology of the wing, body and tail. In addition to being used in a comparison of morphometric methods, geometric morphometrics were used to detect interspecies differences in shape changes.

Geometric morphometrics yielded improved species discrimination relative to traditional methods. The predicted shape variation in the between-group principal components revealed that the largest differences among species lay in the extent to which the wing reaches in the direction of the head. This strong trend in interspecific shape variation is associated with size, which was interpreted as a consequence of evolutionary allometry.

Altogether, this dissertation sheds new light on the processes that might enable the two *Myotis* species and the five *Rhinolophus* species tested to coexist in the same regions without too much competition. In both ensembles, the role of smaller overall size, and especially a shorter body length, a longer tail (independent from overall size) and larger tail area played a major role in improved manoeuvrability performance. We used improved methods compared to older studies, and with regard to external morphological measures, geometric morphometrics was introduced as a better method for finding differences in the shape of the wing, body and tail. In both studied ensembles, similar morphological traits were found to have an impact on manoeuvrability performance, and we found new shape differences among the species. Not only can these results help with better discrimination of other bat species and their niches, but these new findings add to a better understanding of bat flight mechanics.

ZUSAMMENFASSUNG

Die Spezialisierung für eine bestimmte ökologische Nische ist ein wichtiger Prozess der es verschiedenen Arten ermöglicht in den selben Gebieten zusammenzuleben. Diese Anpassungen für verschiedene ökologische Nischen können verhindern, dass eine Art durch eine andere Art dezimiert oder verdrängt wird, wodurch die Koexistenz beider Arten stabilisiert wird. Berühmte Beispiele für die Anpassung an bestimmte Nischen sind die Darwin Finken als auch die Buntbarsche (Cichliden). Bei vielen Fledermausarten wurden ebenfalls Anpassungen an bestimmte Nischen gefunden, z.B. bei der Habitatnutzung, den Aktivitätsmustern während der Nahrungssuche, bei der Echoortung, der Körpergrösse und der Flügelform. In manchen, nahe verwandten und sehr ähnlichen Fledermausarten sind die Anpassungen an bestimmte Nischen allerdings klein und daher schwierig zu erkennen.

Diese Doktorarbeit befasst sich mit zwei Gruppen (Familie der Glattnasen und Familie der Hufeisennasen) solch ähnlicher und schwer zu unterscheidender Fledermausarten, welche sympatrisch in manchen Regionen innerhalb Europas vorkommen. Von den Arten beider Gruppen ist bekannt, dass sie nahe oder in Vegetation nach Beute jagen. In diesem Habitat ist eine hohe Manövrierfähigkeit für eine erfolgreiche Jagd und das Verhindern von Kollisionen mit Vegetation unabdingbar. Es wurde daher die Hypothese aufgestellt, dass die Unterteilung in ökologische Nischen bei den untersuchten Arten durch unterschiedliche Morphologie und hiermit zusammenhängend durch unterschiedliche Manövrierfähigkeit in Vegetation, gewährleistet wird.

Hierzu wurde zunächst die äussere Morphologie der Arten nach Anpassungen an ihre Nahrungssuchstrategie und Unterschiede zu anderen Arten untersucht. Dies wurde mit Hilfe von Flügelbildern (standardisierte Bilder von Tieren mit ausgestreckten Flügeln) gemacht. Von den Flügelbildern wurden anschliessend verschiedene Messungen innerhalb der Flügel-, Körper- und Schwanzregion durchgeführt und grössenunabhängige Verhältniswerte (Ratios) berechnet. Zusätzlich wurde getestet wie gut die Arten in künstlicher Vegetation fliegen können. Hierfür wurde die Manövrierfähigkeit der Arten gemessen. Die verschieden dichte Vegetation (wie z.B. Bäume oder Büsche in einem Wald) wurde mit einem Hindernisparcours mit unterschiedlichen Hindernisabständen

simuliert. Schliesslich wurde die Manövrierfähigkeit der verschiedenen Arten mit den morphometrischen Messungen verglichen.

Die erste getestete Gruppe bestand aus den zwei Mausohrarten Grosses Mausohr (*Myotis myotis*) und kleines Mausohr (*Myotis blythii*). Beide Arten gehören zur Familie der Glattnasenfledermäuse (Chiroptera, Vespertillionidae). Es konnte aufgezeigt werden, dass sich die zwei Arten vor allen Dingen in ihrer Gesamtgrösse, der Flügelspitzenform und der Schwanzlänge relativ zur Körperlänge unterschieden. Das kleine Mausohr erbrachte eine bessere Leistung (weniger Hindernisberührungen oder Landungen) im Hindernisparcours und wurde daher als manövrierfähiger erachtet. Obwohl Unterschiede in der Flügelspitzenform gefunden wurden, war die in Relation zur Körperlänge gesetzte Schwanzlänge das wichtigste Merkmal welches die Manövrierfähigkeit in beiden Arten beeinflusste. Zusätzlich wurden auch zwei Mausohren mit Flügelverletzungen auf ihre Manövrierfähigkeit getestet. Erstaunlicherweise konnten keine Unterschiede zu Ihren Artgenossen aufzeigten werden. Dies war sogar bei einem verletzten Tier mit asymmetrischer Flügelform der Fall. Zusammenfassend wurde daher gefolgert, dass die morphologischen Unterschiede zwischen den zwei Mausohrarten in Bezug zu ihrer Gesamtgrösse, jedoch noch wichtiger zu ihrer Schwanzlänge relativ zur Körperlänge die wichtigsten körperlichen Faktoren sind, welche eine Anpassung an unterschiedliche Nahrungssuch- und Beutefangmethoden bewirken.

Die zweite untersuchte Gruppe bestand aus den fünf verschiedenen Europäischen Hufeisennasenarten: der kleinen Hufeisennase (*Rhinolophus hipposideros*), Blasius Hufeisennase (*Rhinolophus blasii*), Mittelmeerhufeisennase (*Rhinolophus euryale*), Mehelyi-Hufeisennase (*Rhinolophus mehelyi*) und der grossen Hufeisennase (*Rhinolophus ferrumequinum*). Diese fünf Arten unterschieden sich hauptsächlich in ihrer Gesamtgrösse, allerdings konnte eine Überlappung innerhalb der drei mittelgrossen Arten (*R. blasii*, *R. euryale* und *R. mehelyi*) für einige morphologischen Messungen (Armflügelfläche, Schwanzlänge, Schwanzfläche, Körperlänge und Körperfläche) festgestellt werden. Kleinere Arten zeigten, mit Ausnahme der Blasius Hufeisennase, generell eine bessere Leistung im Hindernisparcours als grössere Arten. Obwohl die Blasius Hufeisennase die zweitkleinste Art ist, zeigte sie nur eine ähnlich gute oder sogar schlechtere

Manövrierleistung als die Mehelyi-Hufeisennase welche am zweitgrössten ist. Im Verlaufe des Experiments verbesserten alle Arten ihre Manövrierleistung, jedoch nicht innerhalb direkt hintereinander folgender Durchgänge. Als die Manövrierleistung mit den morphometrischen Messungen in Verbindung gesetzt wurde, stellte sich heraus, dass die Gesamtgrösse, vor allen Dingen hierbei eine kürzere Körperlänge, die wichtigste Rolle für eine bessere Manövrierbarkeit spielt.

Zusammenfassend konnte gezeigt werden, dass bezüglich der Manövrierbarkeit die kleinste Art, als auch die mittelgrosse Mittelmeerhufeisennase am besten an Nahrungssuche in dichter Vegetation angepasst sind. Die Blasius Hufeisennase, die Mehelyi-Hufeisennase und auch die grosse Hufeisennase sind weniger gut an die Nahrungssuche in dichter Vegetation angepasst. Diese Ergebnisse deuten darauf hin, dass die untersuchten Arten sich in ihrer Manövrierfähigkeit gut an verschiedene Mikrohabitate innerhalb der Regionen in denen sie gemeinsam vorkommen angepasst haben. Jedoch sind die Unterschiede zwischen den Hufeisennasen klein und im Vergleich zu anderen Arten sind alle Hufeisennasen sehr gut daran angepasst in Vegetation zu jagen. Im gleichen Hindernisparcours Experiment mit Mausohren (erste untersuchte Gruppe) zeigten beide Arten eine schlechtere Manövrierfähigkeit als alle fünf Hufeisennasenarten.

Schliesslich wurden die verschiedenen, bekannten morphometrischen Methoden (traditionelle Morphometrie) bezüglich der Flügel-, Körper- und Schwanzregion näher betrachtet, sowie auf ihre Fähigkeit der Artunterscheidung verglichen. Zusätzlich zu den bereits bekannten Methoden wurde eine neuere, bisher in Bezug auf externe Fledermausmorphologie, kaum benutzte Methode einbezogen: die geometrische Morphometrie. Die geometrische Morphometrie wird zunehmend in der biologischen Wissenschaft dazu verwendet, Formunterschiede zwischen Arten und Populationen festzustellen. Hier wurde nun untersucht inwieweit die traditionellen morphometrischen Methoden im Vergleich zur geometrischen Morphometrie dazu benutzt werden können korrekte Artunterscheidungen der fünf Europäischen Hufeisennasen anhand der Flügel-, Körper- und Schwanzregion durchzuführen. Zusätzlich zum Methodenvergleich wurde die geometrische Morphometrie dazu benutzt weitere zwischenartliche Formunterschiede zu finden.

Die geometrisch morphometrische Methode resultierte in einer deutlich besseren Artunterscheidung als die traditionellen morphometrischen Methoden. Die Extremform für die Variation entlang der wichtigsten Zwischengruppen-Hauptkomponenten (between group principal components) zeigte auf, dass die grössten Unterschiede zwischen den fünf Hufeisennasenarten in der Reichweite der Flügel in Kopfrichtung zu finden waren. Dieser starke Trend der Flügelreichweite in Kopfrichtung war assoziiert mit der Gesamtkörpergrösse und wurde interpretiert als eine Folge von evolutionärer Allometrie.

In dieser Dissertation wurden neue Erkenntnisse zu den Prozessen entdeckt, welche die Koexistenz der untersuchten Mausohren und Hufeisennasen in denselben Regionen ermöglichen. In beiden Gruppen spielte die geringere Gesamtkörpergrösse, insbesondere die kürzere Körperlänge, eine wichtige Rolle für bessere Manövrierbarkeit. Zusätzlich, unabhängig von der Körpergrösse schien ein längerer Schwanz bzw. eine grössere Schwanzflughautfläche die Manövrierbarkeit zu verbessern. Die verwendeten morphometrischen Methoden in Bezug zur äusseren Morphologie wurden im Vergleich zu älteren Studien verbessert und ein neuerer Ansatz (geometrische Morphometrie) als bessere Methode eingeführt, um Formunterschiede zu finden. Da in beiden Gruppen ähnliche morphometrische Eigenschaften eine Rolle für eine verbesserte Manövrierfähigkeit zu spielen scheinen und neue Formunterschiede bei den untersuchten Hufeisennasen gefunden wurden, können diese Methoden und Ergebnisse nicht nur für zukünftige Studien zur Artunterscheidung und Nischendifferenzierung hilfreich sein, sondern helfen zusätzlich die Flugmechanik von Fledermäusen besser zu verstehen.

2. General Introduction

Ecology is the scientific study of the abundance, distribution and interactions of living organisms and their environment (Leibold et al., 2004). One main goal of ecology is to understand the processes that facilitate species coexistence. One of the most important processes enabling coexistence of different species is the partitioning of niches (Schoener, 1974; Leibold and Mc Peek, 2006). The term niche dates back to the beginning of the twentieth century when Grinnell (1917) used this term to describe the specific habitat requirements and behavioural adaptations of a bird species. Later, niche was defined by Hutchinson (1957) as a multidimensional set of biotic (e.g., predation, competition, parasites and food availability) and abiotic (e.g., climate, sunlight, altitude and wind) conditions in which a given species is able to exist. The partitioning of such niches can prevent the out-competition of one species by another species and therefore stabilizes coexistence (Chesson, 2000; Levine and HilleRisLambers, 2009). The process of species differentiation and specialization towards different occupied niches, and therefore reduced competition, generally takes place over long time periods, encompasses several dimensions and spatial scales and is, therefore, often difficult to prove or disprove (Brennan, 1988; Goldberg, 2006; Grant, 1972; Schoener 1974).

One famous example for the partitioning of niches among closely related species are the Darwin finches (Darwin, 1859). Currently, about fifteen Darwin finch species are known to occur on the Galápagos Islands (Grant and Grant 2008). Their beaks are different in size and shape (a form of character displacement) and enable the species to feed on different food sources (Darwin, 1859; Grant and Grant, 2008). Another nice example includes the cichlid fishes of the African lakes. Approximately 1000 cichlid species can be found alone in Lake Malawi (Kornfield and Smith, 2000). These fish are very diverse in morphology and colouration, and forage in different microhabitats on different foods (Kornfield and Smith, 2000; Meyer et al., 1990; Seehausen, 2006).

In bats, many adaptations to niche partitioning have been found as well. Most bat species forage in specific habitat structures, such as open space, edge space (near the edges of buildings and vegetation or above the ground and water surfaces) or cluttered / narrow space (on or very near to dense structured vegetation or the ground) (Neuweiler, 1984; Aldridge, 1985; Denzinger and Schnitzler, 2013) (Fig. 2.1.).

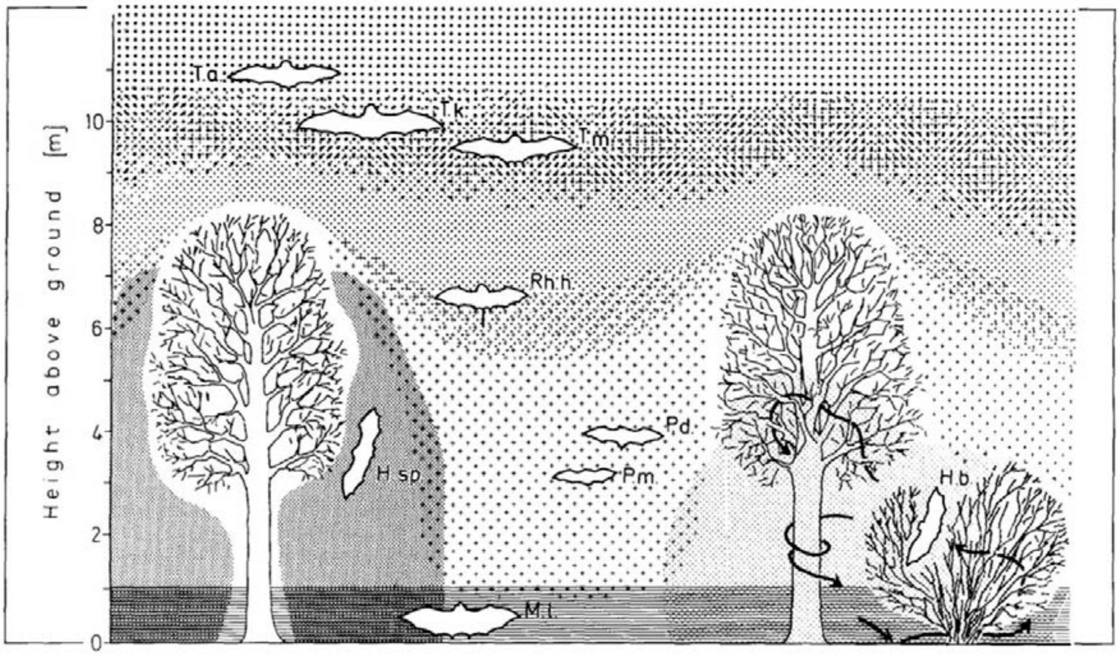

Figure 2.1: Foraging zones of differing bat species in a region within India. The open space foraging zone is used by *Tadaria aegyptiaca* (T.a.), *Taphozous kachhensis* (T.k.), *Taphozous melanopogon* (T.m.), *Rhinopoma hardwickei* (R.h.), *Pipistrellus dormeri* (P.d.) and *Pipistrellus mimus* (P.m.). The edge space and cluttered space zone is used by *Hipposideros speoris* (H.sp.) and *Hipposideros bicolor* (H.b., arrows indicate typical flight paths for this species) and the ground gleaning species *Megaderma lyra* (M.l.). *From Neuweiler 1984.*

Bats have evolved adaptations to different habitats with their echolocation signals, e.g., constant frequency (CF) signals, frequency-modulated signals (FM) or signals with FM and CF components (Neuweiler, 1984; Schnitzler and Kalko, 2001; Siemers and Schnitzler, 2004; Denzinger and Schnitzler, 2013). Bats have also evolved different foraging modes, such as foraging in aerial mode, trawling mode and within or close to vegetation: foraging in flutter detecting mode, passive gleaning mode or active gleaning mode (Denzinger and Schnitzler, 2013). On the basis of the different foraging areas, foraging modes and diets can all bats be classified to seven different guilds (Denzinger and Schnitzler, 2013). Differences in foraging activity patterns can also enable the partitioning of a niche (Emrich et al., 2014). Furthermore, differences in size or wing shape were found to be adaptations to specific niches in the sense that bats foraging in open space are generally larger with narrow wings, high wing loadings and pointed wing-tips, and bats foraging close to vegetation are generally smaller with broader wings, more rounded wingtips and low wing loadings (Alridge and Rautenbach, 1987; Norberg and Rayner, 1987; Norberg, 1994) (Fig. 2.2).

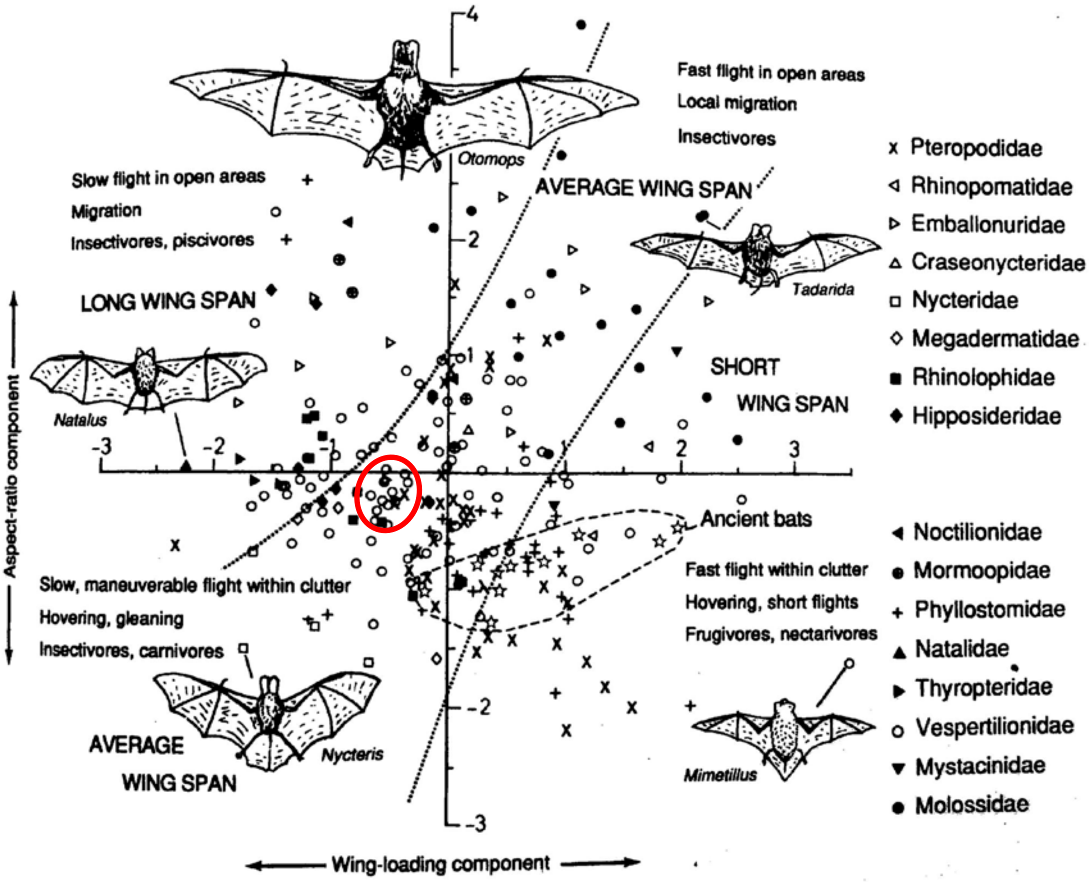

Figure 2.2: The figure shows a scatter plot of the second and third components of a multivariate analysis of wing morphology in a wide range of bat species from 16 families. The second component is correlated with wing loading [(body mass)/(wing area)], and the third component is correlated with aspect ratio [(wing span)^2/(wing area)]. This study shows nicely how wing shapes are correlated to specific flight behaviours and specific habitats. *From Norberg, 1994.* The additional red circle indicates an example for a region within the plot where several species of the family Vespertilionidae have very similar values for aspect ratio and wing loading.

However, in some cases, bat species are very similar although they appear in the same regions. Often, these similar species are closely related to each other; that is, they belong to the same family (see red circle in Fig. 2.2 as an example). In these cases, adaptations for the partitioning of niches are small and therefore harder to find (Saunders and Barclay, 1992; Jacobs et al., 2007; Jacobs and Barclay, 2009; Krueger et al., 2014).

GENERAL AIM

The general aim of this dissertation was to focus on similar and closely related species that co-occur sympatrically and to study under controlled and standardized conditions the ways in which adaptations for a foraging strategy may differ within ensembles of bats. Investigating how such similar species differ is important to an understanding of the processes that encourage coexistence and the specialization to foraging niches.

The focus was specific to bat species that are specialized to forage on or close to vegetation. In this type of habitat, foraging bats face sensorial and mechanical challenges. The prey echoes in open space that are easily recognizable can get overlapped or masked by echoes from vegetation in narrow spaces (Schnitzler and Kalko, 2001; Denzinger and Schnitzler, 2013). The echolocation system of bats specialized to cluttered environment is highly sophisticated for detecting prey (Emde and Menne 1989, Kingston et al., 1999; Schmieder et al., 2012; Denzinger and Schnitzler, 2013), or other cues, such as passive listening are used for this task (Kulzer et al., 1984; Arlettaz et al., 2001; Page & Ryan 2005; Siemers & Swift 2006; Goerlitz et al., 2008; Jones et al. 2011). On the other hand, slow, manoeuvrable flight is essential for effective prey pursuit and avoiding collisions with vegetation. Previous studies have shown that bat species of smaller size or lower mass (low wing loading) can forage in more complex environments and are more manoeuvrable (Alridge and Rautenbach, 1987; Stockwell, 2001; Sleep and Brigham, 2003). In addition to the usually short wings with rounded wing-tips, broad wings and a large tail membrane known to improve the ability to make rapid changes of flight direction result in better manoeuvrability (Norberg, 1994; Dudley, 2002; Gardiner et al., 2011a; Gardiner et al., 2011b; Adams et al., 2012).

In the past, many studies dealt with the sensorial and mechanical problems bats face close to obstacles, such as vegetation. In many cases, the performance of bats was tested with help of obstacle courses. The use of obstacle courses has a long tradition in bat research. More than 200 years ago, Spallanzani showed that bats can avoid strings in total darkness (Galambos, 1942). In 1920 and 1941, Hartridge and Griffin defined ultrasonic waves as the way bats orientate during their flight (Hartridge, 1920; Griffin and Galambos, 1941). While Hartridge described bat flight during many different situations, Griffin presented vertically suspended wires in a standardized way. From 1960 onwards, several scientists focused on the sensory basis of string detection (Schneider and Mohres, 1960; Schnitzler, 1968; Mogdans et al., 1988). In 1985, Aldridge began experiments, mainly on the manoeuvrability of British bats. He showed that the manoeuvrability of the tested species played a major role in the selection of their foraging sites. Therefore, he stated that the choice of foraging site is dependent on the animal's ability to fly and on its ability to detect prey within that site (Aldridge, 1985). For his manoeuvrability experiments, Aldridge used strings (binder twine), which were suspended vertically in several rows (Aldridge, 1985; Alridge and Rautenbach, 1987). Since that time, many other scientists have conducted obstacle experiments with different bat species (Jones et al., 1993; Rhodes, 1995; Kingston, 2001; Stockwell, 2001; Barber et al., 2003; Waters and Vollrath, 2003; Barchi et al., 2013; Falk et al., 2014; Sandig et al., 2014).

Researchers who have conducted experiments with obstacle courses have encountered some challenges. First, if someone wants to compare former obstacle experiments, there may be problems with comparing the results obtained by different investigators because the enclosures, the type of obstacles and the number and position of the obstacles (e.g., only one or several obstacle rows) varied. Second, it may be difficult or impossible to distinguish between the limits to an animal's ability to perform a particular flight task as being attributable to sensory or mechanical constraints (Dumont et al., 2009).

In this dissertation, I focused on two ensembles of bat species of the family Vespertilionidae and the family Rhinolophidae that are adapted to forage close to vegetation. An ensemble is defined as a phylogenetically restricted group of species that exploits similar resources within a community (Fauth et al., 1996). Both ensembles were tested in similar obstacle courses for their mechanical performance by measuring the bats' manoeuvrability. To rule out sensory challenges, the obstacles were designed to be easily detectable for the bats (Fig. 2.3 A and B).

In addition to the flight performance, external morphology measurements of the studied species were compared for adaptations to the species' foraging styles, for differences among species and, most importantly, in combination with the obstacle course experiments to determine the influence of morphology on manoeuvrability. In the course of this dissertation, I recognized the need to further develop the external morphology measures for bats; therefore, the methods changed slightly (see the example of wing pictures Fig. 2.3 C and D) in comparison to former studies, and the last chapter deals only with morphological measurements.

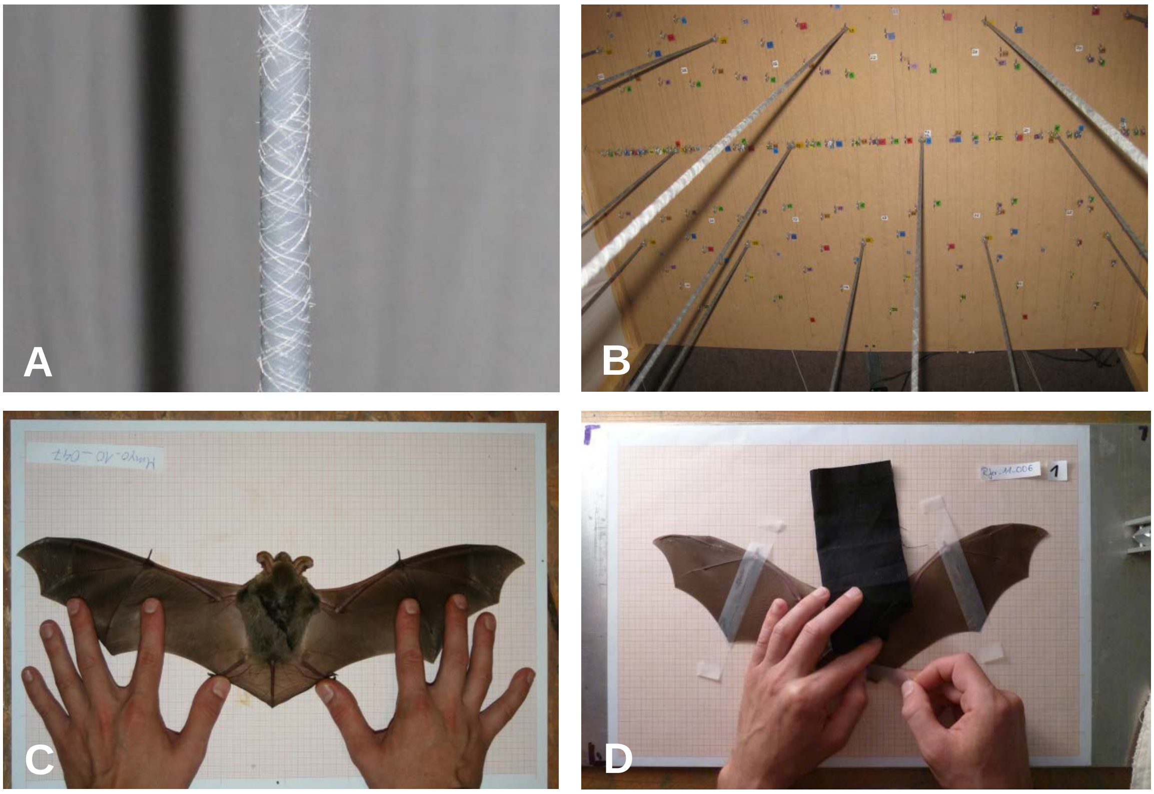

Figure 2.3: Set up for obstacle avoidance experiment (a-b) and external measurements (c-d): a) Close-up of one obstacle; b) View of obstacle course from below; The differently coloured markings indicate the testing of differing obstacle spacing; c) Wing picture of a *Myotis myotis*; d) Fixing a Rhinolophid bat with a specific type of tape when taking wing pictures. The head of the bat was covered before taking the photographs to minimize the disturbance from the light.

The focus ensembles consisted of two mouse-eared bat species (Fig. 2.4 and 2.5) belonging to the guild of passive gleaning foragers and all five European horseshoe bat species (Fig. 2.6) belonging to the guild of flutter detecting foragers. These two ensembles were chosen because, besides the similarities of the included species within each group, the ecology of the species had already been studied in several field studies. Therefore, it was possible to compare earlier results with the results of the experiments conducted under laboratory conditions for this dissertation. The species did not occur sympatrically in all regions in Europe; however, in south-eastern Europe, this was the case. The study site for all experiments was therefore located in Bulgaria, where all species occur in higher numbers.

Figure 2.4: Greater mouse-eared bat (*Myotis myotis*) after experiments and during release.

Figure 2.5: Lesser mouse-eared bat (*Myotis blythii*).

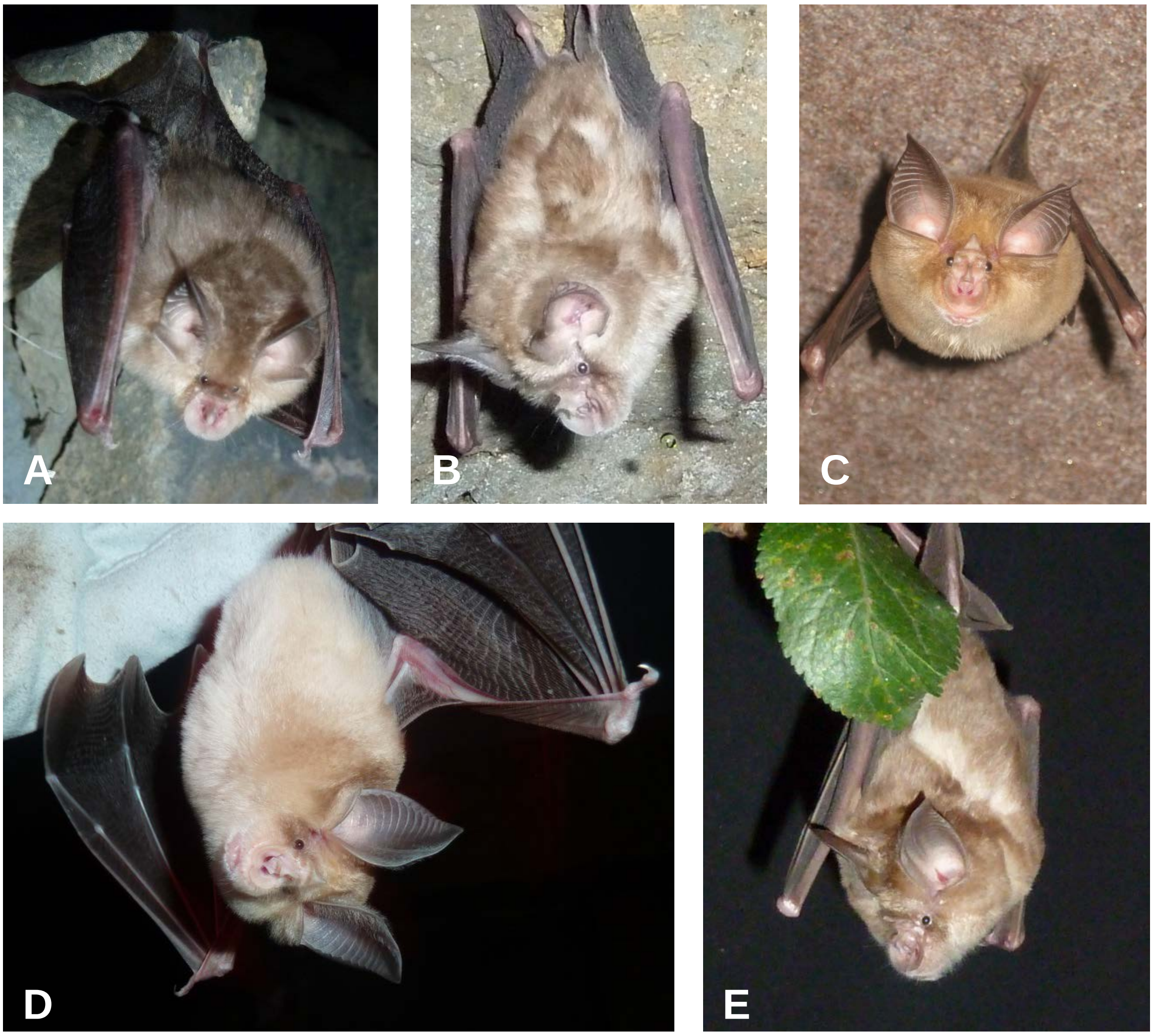

Figure 2.6: European horseshoe bats: A) *Rhinolophus hipposideros*, B) *R. blasii*, C) *R. euryale*, D) *R. mehelyi*, E) *R. ferrumequinum*.

3. Manoeuvrability in mouse-eared bats

The Tail plays a major role in the differing manoeuvrability of two sibling species of mouse-eared bats (*Myotis myotis* and *Myotis blythii*)

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ABSTRACT

Two sympatrically occurring bat species, *Myotis myotis,* Borkhausen 1797, and *Myotis blythii,* Tomes 1857 (Chiroptera, Vespertillionidae), share numerous similarities in morphology, roosting behaviour and echolocation and are often difficult to distinguish. However, despite these similarities, their foraging behaviour is noticeably different. Our aim was to examine the extent to which these different foraging strategies reflect morphological adaptation. We assessed whether the morphology of the wing, body and tail differed between *M. myotis* and *M. blythii*. In addition, in a laboratory experiment involving an obstacle course, we compared differences in manoeuvrability by relating them to our morphological measurements.

The two species differed in their overall size, wing-tip shape and tail-to-body length ratio. The generally smaller-sized *M. blythii* performed better in the obstacle course and was therefore considered to be more manoeuvrable. Although differences in wing-tip shape were observed, we found the most important characteristic affecting manoeuvrability in both species to be the tail-to-body length ratio. Additionally, when we compared two bats with injured wing membranes with unharmed bats of the same species, we found no difference in manoeuvrability, even when the wing shape was asymmetric. We therefore postulate that morphometric differences between the two species in their overall size and, more importantly, in their tail-to-body length ratio are the main physical characteristics providing proof of adaptation to different foraging and feeding strategies.

INTRODUCTION

In the wild, populations of closely related and similar species can often be seen foraging in the same habitats. Famous examples include the Darwin finches (Darwin, 1859) and East African cichlid fishes (Meyer et al., 1990). At first glimpse, these closely related species might appear to be in strong competition with each other, but specializations can usually be detected in their feeding niches (Schluter, 2001). For flying animals, such as bats or birds, the ability to perform manoeuvres is crucial in avoiding obstacles and approaching prey in dense vegetation (Findley et al., 1972; Norberg and Rayner, 1987; Norberg, 1994). Differences in manoeuvrability have generally been considered to be an important factor in niche differentiation and, ultimately, to represent a fundamental process underlying the adaptive radiation of bats, which are the second most species-rich mammalian order (Aldridge, 1985; Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Voigt and Holderied, 2012). In the context of flight, manoeuvrability is usually defined as the minimum radius of a turn an animal can achieve without reducing its speed. Another term related to aerial manoeuvres is agility, which is defined as the maximum roll acceleration during the initiation of a turn and is therefore measured based on the rapidity with which a flight path can be altered (Norberg and Rayner, 1987; Norberg, 1994). In an ecological context, the term manoeuvrability refers to the ability to fly through limited space while avoiding vegetation or other obstacles. Thus, our definition of manoeuvrability includes agility.

Chiroptera encompasses over 1230 species distributed worldwide (Schipper et al., 2008; Kunz et al., 2011) exhibiting diverse wing morphologies and, in the case of species using echolocation for orientation, differentiation in echo-acoustic characteristics (Neuweiler, 1984; Schnitzler and Kalko, 2001). While we can predict that morphologically and echo-acoustically different species will use and perceive the world in different ways, there are examples of species that co-exist in the same environment despite showing little or no measurable morphological or echo-acoustical difference. It is not obvious how these species can coexist without strong competition (Saunders and Barclay, 1992; Arlettaz, 1999; Jacobs and Barclay, 2009; Salsamendi et al., 2012a). Investigating how these morphologically similar and closely related species adapt to different feeding and foraging niches should lead to a better understanding of the mechanisms that encourage coexistence and foraging niche specialization.

The European greater mouse-eared bat (*Myotis myotis*, Borkhausen 1797) and the lesser mouse-eared bat (*Myotis blythii*, Tomes 1857) can sometimes be found roosting together and even occasionally hybridising (Berthier et al., 2006). Regarding morphology, *M. myotis* is very similar to *M. blythii* in general, though it is usually slightly larger than *M. blythii* (Arlettaz et al., 1991; Dietz et al., 2009). The echolocation calls of these species are similar, and although differences occur, none of them appear to be consistent among all individuals. Thus, distinguishing these two species in the field is difficult (Russo and Jones, 2002; Walters et al., 2012). However, despite their morphological and acoustic similarities, these two species show behavioural divergence in foraging behaviour (Arlettaz, 1996, 1999; Berthier et al., 2006; Siemers et al., 2011). *M. myotis* prefers habitats with open accessible ground, in contrast to *M. blythii*, which typically hunts in long grass meadows (Arlettaz, 1996; Güttinger et al., 1998; Arlettaz, 1999; Rainho et al., 2010; Siemers et al., 2011). Our purpose was first to more closely examine the wing, body and tail morphology of these two species to determine whether they actually differ only in their overall size, or if differences in shape exist as well. We expected that all of the length and area measurements of the wing, body and tail of *M. blythii* would present significantly lower values compared with *M. myotis*. In other words, we expected to find that the main difference between these species is due to their different sizes and that the two species are simply scaled versions of the same model (Norberg and Rayner, 1987; Stockwell, 2001; Andreas et al., 2013). Then, in a second step we sought to ascertain whether the differences in the ecology of the two species could be attributed to morphological adaptations, followed by differences in flight performance. Arlettaz (1996) speculated that *M. blythii* gleans insects directly from grass stalks, which would require greater manoeuvrability than *M. myotis*, which lands on the ground to catch prey (Arlettaz, 1999). We followed these suggestions and hypothesised that *M. blythii* is morphologically better adapted than *M. myotis* to slow, manoeuvrable flight, close to or within dense ground vegetation (e.g., long grass meadows, steppe-like areas). We assumed that the smaller wing span of *M. blythii* would play a major role in making the species more manoeuvrable than *M. myotis* (Norberg, 1994; Stockwell, 2001). However, in cases where the observed morphological differences deviate from the overall size, we assumed that the two species would differ in a way that is consistent with *M. blythii* being more manoeuvrable, for example, presenting a wing shape with more pointed wing-tips (Findley et al., 1972; Norberg and Rayner, 1987; Norberg, 1994). To test our hypotheses, we compared wing, body and tail measurements of the two sibling species as well as size-independent wing shape ratios and indices (Norberg and Rayner, 1987; Norberg, 1994) (Table 3.1).

Table 3.1: Overview of the predictions, results and findings with regard to morphology and manoeuvrability of the greater and lesser mouse-eared bat (*Myotis myotis*, Borkhausen 1797 and *M. blythii*, Tomes 1857).

Variable	Prediction morphology	Result morphology	Interpretation morphology	Prediction manoeuvrability	Results manoeuvrability	Interpretation manoeuvrability
Mass	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	Contrary to preditcions overall size differences in these variables do not play an important role for manoeuvrability differences
Wing span	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	strong effect	**No effect**	
Wing area	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	
Hand-wing length	*M. myotis* > *M. blythii*	***M. myotis* = *M. blythii***	Hand-wing relatively to size longer in *M. blythii* because of differing wing-tip shape	Effect	**No effect**	
Hand-wing area	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	
Arm-wing length	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	
Arm-wing area	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	
Forearm length	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	Effect	**No effect**	
Tail length	*M. myotis* > *M. blythii*	***M. myotis* = *M. blythii***	Tail shape differs between species	No effect	**Strong effect**	Longer tail increases manoeuvrability
tail membrane area	*M. myotis* > *M. blythii*	***M. myotis* = *M. blythii***	Tail shape differs between species	No effect	No effect	might play a role as well, but effect was not found
Body length	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	No effect	**Strong effect**	Important variable standing in line with overall size differences
Body width	*M. myotis* > *M. blythii*	*M. myotis* > *M. blythii*	Overall size difference	No effect	No effect	No importance found
Body area	*M. myotis* > *M. blythii*	***M. myotis* = *M. blythii***	Body of *M. blythii* more compact	No effect	No effect	No importance found
Aspect ratio	*M. myotis* ≥ *M. blythii*	*M. myotis* = *M. blythii*	No difference between species	Effect	Effect	Of general importance for manoeuvrability
rel. wing loading	*M. myotis* ≥ *M. blythii*	*M. myotis* = *M. blythii*	No difference between species	Effect	Effect	Of general importance for manoeuvrability
Tip length ratio	*M. myotis* ≥ *M. blythii*	***M. myotis* < *M. blythii***	*M. blythii* has relatively to size a longer hand-wing	Effect	**No effect**	Against our predictions does wing tip shape not play a role
Tip area ratio	*M. myotis* ≤ *M. blythii*	*M. myotis* = *M. blythii*	No difference between species	Effect	Effect	Generally important but independent from the wing shape
Wing-tip shape index	*M. myotis* ≥ *M. blythii*	*M. myotis* > *M. blythii*	Wing-tip of *M. blythii* is more pointed than wing-tip of *M. myotis*	Strong effect	**No effect**	Against our predictions does wing tip shape not play a role
tail-to-body length ratio	*M. myotis* = *M. blythii*	***M. myotis* < *M. blythii***	Tail of *M. blythii* is relatively to size longer than the tail of *M. myotis*	No effect	**Strong effect**	Longer tail increases manoeuvrability and in relation to overall size is most important variable for species differences
Tail-to-wing area ratio	*M. myotis* = *M. blythii*	*M. myotis* = *M. blythii*	Tail area might be larger, with used method tail membrane area was possibly not fully extended	No effect	No effect	Might play a role as well, but effect was not found

The results column indicates found or not found significant differences between the species in regard to differences in morphology or manoeuvrability (best three models for unintended landings). In case significant differences were found for the morphology the sign < or > indicates if the values were smaller or larger for *M. myotis*. The column interpretation gives a short explanation how the results column can be interpreted for the studied species. Results that were against our predictions are shown in bold type.

Moreover, we evaluated the manoeuvrability of both species in a standardised vegetation-like obstacle course (Aldridge, 1985; Rhodes, 1995; Stockwell, 2001).The results obtained from the obstacle course were first tested for variability between species. Second, we removed the species variable and instead included the morphological variables to assess how these parameters contributed to performance differences.

MATERIALS AND METHODS

Animals

We caught bats using a harp trap (Faunatech, Victoria, Australia) or mist nets (Ecotone, Sopot, Poland) at the entrance of caves in northeastern Bulgaria from the beginning of May until the end of August 2010. The capture and husbandry of the bats and behavioural analyses were carried out under license from the official Bulgarian authorities (MOEWSofia and RIOSV-Ruse, permit numbers 193/01.04.2009 and 205/29.05.2009) in accordance with recommendations of the Canadian Council on Animal Care for bats and the EUROBATS Resolution 6.5.

We identified the two species based on differences in forearm length (*M. myotis*: 5.5 - 6.7 cm and *M. blythii*: 5.1 - 6.2 cm) and upper tooth row length (caninus to the 3rd molar, *M. myotis*: 0.92 to 1.06 cm and *M. blythii*: 0.81 to 0.95 cm) (Dietz et al., 2009). Individuals of intermediate size (forearm length between 6.0 - 6.2 cm) were not used in the experiments because they could not be precisely identified to the species level. All of the bats were sexed and weighed (electronic scale, Kern, EMB 500-1, $d = 0.1$ g, Balingen, Germany), and further standard measurements (body length, wing span, tail length) were obtained with a dial calliper (Measy 2000 calliper, Switzerland, precision 0.1 mm) (Fig. 3.1 B). Only adult bats were used for the experiments.

The bats were retained for a maximum of 10 days at the Tabachka Bat Research Station (Bulgaria) of the Sensory Ecology Group (Max Planck Institute for Ornithology, Seewiesen, Germany). Up to six bats were housed together in a cage (49 cm length x 37 cm height x 35 cm breadth) at 20 °C ± 3 °C under a 14/10 h light/dark photoperiod. The bats were fed mealworms (*Tenebrio molitor*, Linnaeus, 1758, larvae, Coleoptera) by hand each night. We measured the body mass of the bats every day to ensure that they maintained their body condition. Water was available *ad libitum*. After the experiments, all of the bats were released in good health at the site of capture.

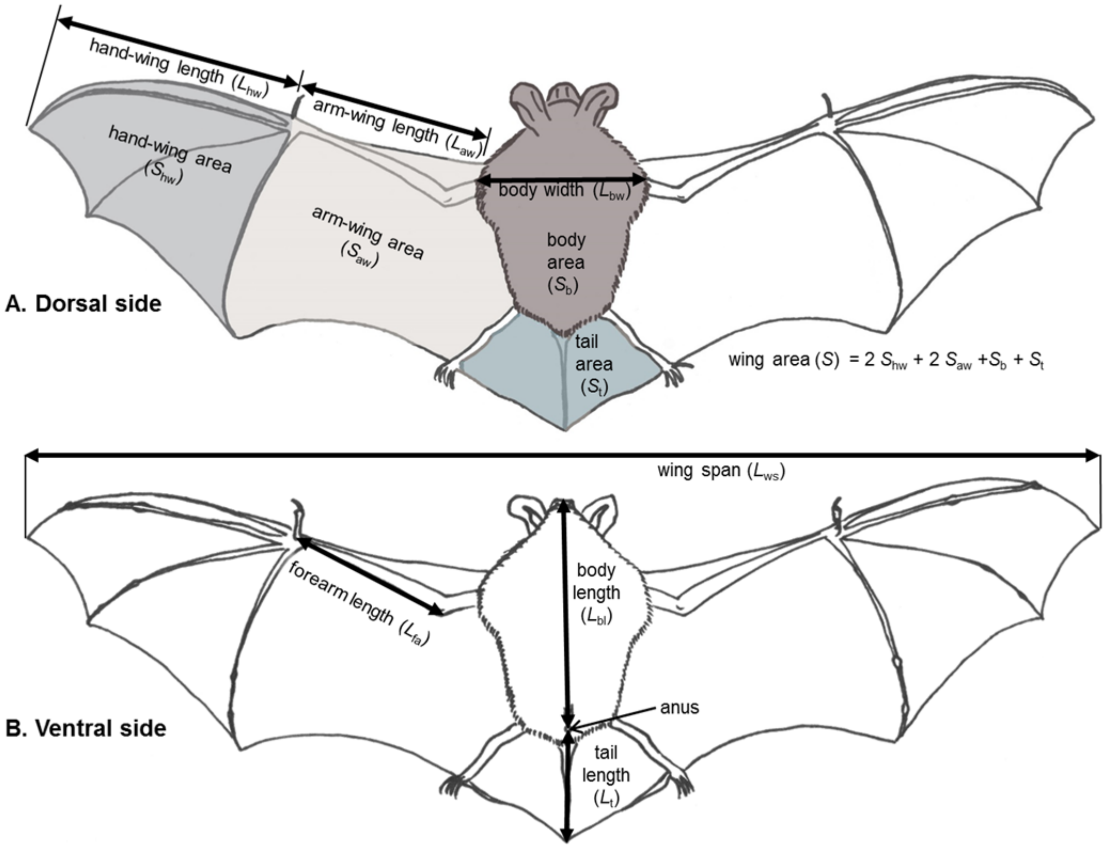

Figure 3.1: Drawing of a greater mouse-eared bat (*Myotis myotis*, Borkhausen 1797), showing all of the morphometric measurements that were taken. The lengths and areas of the wing, body and tail elements were measured from wing pictures taken from the dorsal side (A). Other measurements were conducted directly by handling the bat (B).

Morphology

In addition to the standard morphological measurements, we took pictures of the wings of 17 male *M. myotis* and 15 male *M. blythii*. We held each bat on a sheet of graph paper and carefully stretched the wings until they were fully extended. We took several digital pictures of each bat from a distance of approximately 1 m. To reduce measurement errors, we analysed three pictures per individual and noted the average values. In each picture, we measured either the left or the right wing depending on which was more stretched based on an estimate of the wing area (wing length x wing width). Using these pictures, we measured the hand-wing length, arm-wing length, hand-wing area (chiropatagium) and arm-wing area (propatagium and plagiopatagium: Fig. 3.1 A) according to Norberg and Rayner (1987). Additionally we measured the tail membrane area (uropatagium), body width and body area. To obtain these measurements, we used the image processing program Image J (version 1.44) (Rasband, 1997-2012).

Using the wing and body measurements and the standard morphological measures, we calculated the aspect ratio, relative wing loading, tip length ratio, tip area ratio, wing-tip shape index (Norberg and Rayner 1987; Norberg 1994), tail-to-body length ratio and tail-to-wing area ratio (Fig. 3.1 and table S 3.1). In the next step, we compared each parameter between the two *Myotis* species with non-parametric Kruskal-Wallis tests in R (version: 3.0.0, 13.04.03) (RcoreTeam, 2013), adjusting the *p*-values for multiple testing with a Bonferroni correction. We employed the nonparametric Kruskal-Wallis tests because the data were not normally distributed among the species populations. The 95 % confidence intervals of the median values for the differing parameters were calculated using the bootstrapping method (type: percentile) (Canty and Ripley, 2013). To reveal the relationships between the obtained parameters, all of the morphological measures, ratios and indices were compared to determine their degree of correlation using a cluster dendrogram in R, in the R package pvclust (Suzuki and Shimodaira, 2006).

Manoeuvrability

From the bats that were used to obtain wing measurements, we randomly chose seven individuals of each species and conducted a manoeuvrability test for each of them in a flight tunnel (8.0 m length x 1.5 m breadth x 2.4 m height). Performance was assessed using an obstacle course consisting of three rows of plastic tubes (grey PVC, length approx. 2.3 m). The obstacles all had a diameter of 6 mm and were detectable using echolocation (Sumer et al., 2009). The surface of the obstacles was roughened to enhance detection. Upon ensonification of the obstacles using a frequency-modulated signal ranging from 120 kHz to 20 kHz, they produced a distinct echo (Fig. S 3.1). For ensonification, we positioned a microphone (Gras microphone 1/8-inch Type 40DP, Holte, Denmark) above a loudspeaker (Ultrasonic Dynamic Speaker ScanSpeak, Avisoft, Berlin, Germany) at a height of 110 cm and placed an obstacle 50 cm in front of the loudspeaker.

The obstacles were suspended from the ceiling on hooks mounted on a wooden plate and fixed on the ground in sand that covered the floor of the experimental room. A wooden plate attached to a frame was placed 4 m away from the entrance to the flight tunnel (Fig. 3.2), and the obstacles were spaced equidistantly within and between the three rows.

The trials took place at night, and before the actual trials began, each bat had a chance to fly through the tunnel once without obstacles to become familiar with the flight tunnel and the procedure. Then, at the beginning of each individual trial, a single bat was hung on a perch. After a period of 3 min, during which the bat could start flying on its own, the perch was gently turned to prompt the bat to fly. After the bat started flying, it was given 3 min to fly through the obstacle course to the end of the flight tunnel. In cases where a bat stopped and perched, it was prompted to continue flying by finger snapping, or it was removed and re-released on the perch at the beginning of the tunnel. The first arrangement tested for *M. myotis* employed spacing of 41 cm between obstacles, while that for *M. blythii* employed 38 cm spacing, corresponding to their average respective wing spans. For all other trials, arrangements with 9 different spacings (19 cm, 21 cm, 29 cm, 31 cm, 38 cm, 41 cm, 44 cm, 48 cm and 52 cm) were presented to the individuals three times each in a pseudo-randomised order. The same spacing was never repeated in successive trials. The manoeuvrability performance of the bats tested during the different trials can therefore be compared to a situation in the wild where the bats are flying in an unknown, cluttered habitat. When a bat completed a flight through the obstacle course (including the first flight without obstacles), it was prevented from flying back and was rewarded with 3 - 5 mealworms. Each individual completed 5 - 8 trials consecutively, with a short break of usually 5 - 10 min between each flight and was tested in this way once or twice per night. The trials were repeated once in the case of technical problems, e.g., if the bat was disturbed by the observer shortly before flying through the course, or if the bat showed certain behaviours (starting from the wall less than 1 m from the obstacle course, touching the wall, crawling or landing without touching an obstacle while flying through the course). The obstacles were flexible and yielded slightly when touched by a bat. Collisions with the obstacles produced no apparent signs of injury, though the bats were checked regularly. Each trial was monitored directly by the observer using a headlamp with a red light, and the trials were recorded for further analysis using four infrared-sensitive cameras (WAT-902H2 Ultimate, Watec, Tsuruoka, Japan) and Digi Protect (Abus, Affing, Germany) surveillance software. Infrared light was provided through a stroboscope (875 nm wavelength, flash rate 50 Hz, i.e., one flash per video half-frame, custom made, University of Tuebingen).

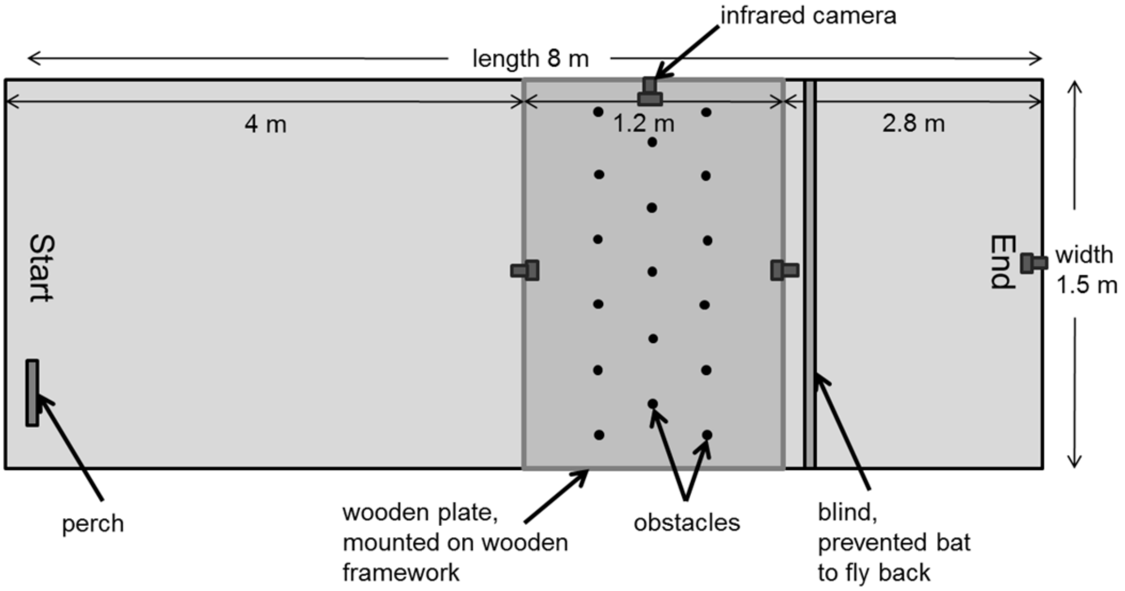

Figure 3.2: Flight tunnel - view from above. Sketch of the experimental set up (not exactly to scale), showing the flight tunnel and the wooden plate in the middle, where the obstacles were positioned. The bats flew from the Start position, through the obstacle course, to the End, where they received a reward.

We assumed that the bats would attempt to avoid touching the obstacles and eventually make an unintended landing on the ground. Under natural circumstances, even slight contact with vegetation might be risky because some branches have spines or thorns that could lead to wing injuries (Davis, 1968). Likewise, unnecessary and uncontrolled landings could increase the risk of injuries and predation (Lima and O'Keefe, 2013). Therefore, using the video recordings for every trial, we counted each time when one of the obstacles was touched and each unintended landing (landing on the ground after touching one or more obstacles) within or shortly behind the obstacle course.

Differences in manoeuvrability between the species

We employed generalized linear mixed effects models (GLMMs) in R (Bates et al., 2013; RcoreTeam, 2013) using the glmer function to check for differences in manoeuvrability between the species. We first tested the differences between species, based on their performance in obstacle avoidance, by comparing the total number of touched obstacles and the number of unintended landings on the ground in a basic GLMM. The basic GLMM included the function of spacing between the obstacles and species identity modelled with a Poisson error distribution, accounting for individual differences and pseudo-replication by using individual identity as a random factor. Furthermore, we tested whether repeated measures for a given spacing had any effect.

Incorporating morphometric variables into the basic GLMM

We examined the assumed species differences more closely for the purpose of determining which morphometric variables best explain the observed species-specific differences. Therefore, the morphological measures and ratios (Table 3.2 and 3.3) obtained for the 14 individuals tested in the obstacle course were added to the same basic GLMM, except that we did not include the species identity in this case. Each time, only one variable was added to the basic model (20 variables = 20 models). All of the measured and calculated morphological variables were included in the modelling process. None of the correlating variables was removed because the fact that two variables are correlated does not guarantee that they both have the same functional importance for the tested manoeuvrability performance. For the added morphometric variables in the differing models, the *p*-values were corrected for multiple testing with a Bonferroni correction factor. The morphological variables and their *p*-values were directly compared and discussed regarding their influence, or lack of thereof, in relation to the manoeuvrability performance.

Forward selection modelling procedure with morphological variables

Subsequently, we tested whether there were minimum adequate models consisting of several important morphological variables that best explained the differences in the manoeuvrability of the tested bats as a function of morphological differences. To obtain the best minimum adequate models, we used the forward selection procedure based on a minimising Bayesian information criterion (BIC). In the first step, we compared the BIC values of the 20 models (each included one morphological variable) with each other and searched for the models with the lowest BIC values. We tested these BIC models further by adding one of the 19 other variables each time. Whenever one of the new models showed a BIC value that was at least two units lower compared with the simpler model, it was considered to be a better model. The modelling process was continued by adding further variables until no better models could be found (Table S 3.2 and S 3.3)

Comparison of individuals with injured wings

During the bat capture events, two individuals with old wing membrane injuries were captured. Because the two bats (one female *M. myotis* and one male *M. blythii*) belonged to our target species and there have been only a few studies on naturally occurring injured bats, we decided to obtain wing pictures for these bats and to test their manoeuvrability performance under the same conditions applied to the other individuals in our obstacle course. We compared the obstacle avoidance behaviour of the injured individuals with the performance of the unharmed conspecifics. Because of the small sample size, we did not carry out statistical testing.

RESULTS

Morphology

Eight of the twelve length and area measurements for the body and wings of the bats and the body mass were significantly greater in the *M. myotis* individuals compared with *M. blythii* (Table 3.1 and 3.2). The tail length (Kruskal-Wallis test, χ^2 (Chi-Square) = 6.49, df = 1, p = 0.141), tail membrane area (Kruskal-Wallis test, χ^2 = 0.032, df = 1, p = 1.0), body area (Kruskal-Wallis test, χ^2 = 4.24, df = 1, p = 0.515) and hand-wing length (Kruskal-Wallis test, χ^2 = 8.02, df = 1, p = 0.060) did not differ significantly between the two species (Table 3.2). In the case of hand-wing length, the difference between the two species tended toward significance, with lower values being found for *M. blythii,* which is consistent with the other length and area measurements. Regarding the body area, most *M. myotis* individuals (N = 12) exhibited higher values than *M. blythii* (N = 9), but the results were not significant (Table 3.2). The 95 % confidence interval of the tail length of *M. myotis* ranged from 5.1 to 5.4 cm (median: 5.2) and that of *M. blythii* from 5.2 to 5.8 cm (median: 5.6). Thus, the tail lengths strongly overlapped, but the median length was greater for the generally smaller species, *M. blythii*. A similar result was found for the tail membrane area (Table 3.2).

Table 3.2: Median values of mass, length and area measurements with 95 % confidence intervals calculated with bootstrapping method in the greater and lesser mouse-eared bat (*Myotis myotis,* Borkhausen 1797 and *M. blythii,* Tomes 1857).

	M. myotis (*N* = 17)	*M. blythii* (*N* = 15)	*p*
Mass [g]	26.19 (25.9 - 27.2)	22.61 (21.8 - 23.9)	< 0.001
Wing span, L_{ws} [cm]	41.3 (40.9 - 42.0)	38.0 (37.5 - 38.7)	< 0.001
Wing area, S [cm²]	255.1 (248.3 - 262.0)	226.8 (215.4 - 233.8)	< 0.001
Hand-wing length, L_{hw} [cm]	9.4 (9.1 - 9.5)	8.9 (8.6 - 9.1)	0.060
Hand-wing area, S_{hw} [cm²]	38.7 (37.9 - 40.4)	33.8 (32.1 - 35.4)	0.008
Arm-wing length, L_{aw} [cm]	7.7 (7.4 - 7.8)	6.8 (6.6 - 7.1)	< 0.001
Arm-wing area, S_{aw} [cm²]	62.3 (59.1 - 64.1)	53.8 (49.7 - 55.1)	< 0.001
Forearm length, L_{fa} [cm]	6.3 (6.2 - 6.3)	5.7 (5.6 - 5.8)	< 0.001
Tail length, L_t [cm]	5.2 (5.1 - 5.4)	5.6 (5.2 - 5.8)	0.141 (ns)
Tail area, S_t [cm²]	20.0 (17.6 - 20.8)	19.8 (17.4 - 22.5)	1.0 (ns)
Body length, L_{bl} [cm]	7.4 (7.2 - 7.6)	6.5 (6.3 - 6.7)	< 0.001
Body width, L_{bw} [cm]	6.6 (6.48 - 6.69)	6.0 (5.6 - 6.2)	< 0.001
Body area, S_b [cm²]	33.7 (31.9 - 36.3)	30.8 (30.4 - 32.2)	0.515 (ns)

N refers to the number of individuals and ns refers to not significant. The values of the two species were compared with a Kruskal-Wallis test (df = 1) and the resulting *p*-values were corrected for multiple comparisons with a Bonferroni correction factor. Significance is indicated by the corrected *p*-values.

Among the size-independent ratios and indices, we found no significant differences regarding the aspect ratio, relative wing loading or tip area ratio (Table 3.1 and 3.3). However, the tip length ratio (Kruskal-Wallis test, $\chi^2 = 12.73$, df = 1, $p = 0.003$) and the wing-tip shape index (Kruskal-Wallis test, $\chi^2 = 7.29$, df = 1, $p = 0.049$) were significantly different.

Table 3.3: Medians of ratios and indices with 95 % confidence intervals in the greater and lesser mouse-eared bat (*Myotis myotis,* Borkhausen 1797 and *M. blythii,* Tomes 1857).

	M. myotis (*N* = 17)	*M. blythii* (*N* = 15)	*p*
Aspect ratio	6.61 (6.50 - 6.86)	6.53 (86.30 - 6.68)	0.510 (ns)
Relative wing loading [N/m²]	34.21 (32.37 - 35.30)	34.92 (33.0 - 37.35)	1.0 (ns)
Tip length ratio	1.23 (1.19 - 1.25)	1.31 (1.27 - 1.34)	0.003
Tip area ratio	0.64 (0.61 - 0.66)	0.63 (0.62 - 0.66)	1.0 (ns)
Wing-tip shape index	1.11 (1.00 - 1.15)	0.93 (0.87 - 1.03)	0.049
Tail-to-body length ratio	0.69 (0.68 - 0.74)	0.84 (0.83 - 0.89)	< 0.001
Tail-to-wing area ratio	0.086 (0.078 - 0.090)	0.095 (0.084 - 0.109)	0.253 (ns)

N refers to the number of individuals. The values of the two species were compared with a Kruskal-Wallis (df = 1) test and the resulting *p*-values were corrected for multiple comparisons with a Bonferroni correction factor. Significance is indicated by the corrected *p*-values.

Because we found no significant differences for tail length and tail membrane area, and none of the size-independent ratios or indices that we calculated were explicitly related to the tail, we decided to define two new ratios: the tail-to-body length ratio and the tail-to-wing area ratio. The tail-to-body length ratio (TB_l) is the ratio of the length of the tail (L_t) to the length of the body (L_{bl}); $TB_l = L_t / L_{bl}$), and the tail-to-wing area ratio (TS_S) is the ratio of the area of the tail membrane (S_t) to the wing area, but without the tail area ($TS_S = S_t / (2\ S_{hw} + 2\ S_{aw} + S_b$). Accordingly, a higher value for the tail-to-body length or tail-to-wing area ratio indicates a longer tail or larger tail area in relation to the body or wing. We found a significantly higher tail-to-body length ratio for *M. blythii* than for *M. myotis* (Kruskal-Wallis test, $\chi^2 = 20.67$, df = 1, $p < 0.001$). For the tail-to-wing area ratio, we did not find a significant difference (Kruskal-Wallis test, $\chi^2 = 4.39$, df = 1, $p = 0.253$).

The cluster dendrogram (Fig. 3.3) presented four clusters, each of which exhibited highly correlated variables. In the case of clusters 6 and 9, the length or area measurements were one of the main elements of the corresponding and highly correlated ratio (tail-to-body length ratio or tail-to-wing area ratio). In cluster 13, both relative variables included the wing area within their formula.

Cluster 14 consisted of the variable mass and all of the length and area measurements, except for the tail length and tail area. Within cluster 14, a clear separation between the morphometric measurements for the same wing, body or tail region was observable in some cases (e.g., body area and body length or body width). Cluster 14 included all of the length and area measurements that were significantly different between *M. myotis* and *M. blythii*, with the exception of the hand-wing length and body area, (Table 3.2). The measurements of cluster 14 all showed greater values for *M. myotis*, and they therefore represent the overall size differences of the two species most clearly.

Manoeuvrability

All seven *M. myotis* and *M. blythii* successfully completed 26 valid trials on average (max. 27 trials, min. 22 trials). We recorded 181 valid trials for *M. myotis* and 182 for *M. blythii* in total. The narrowest obstacle spacing that a *M. blythii* individual was able to negotiate without touching any obstacle was 29 cm (average min. negotiated spacing: 43.1 cm, *N* = 7) (Video S 1[1]). For *M. myotis*, the narrowest spacing that could be negotiated without any touches or unintended landings was 38 cm (average min. negotiated spacing: 44.7 cm, *N* = 6). One *M. myotis* did not manage to fly through the course without any touches, even at the broadest spacing of 52 cm.

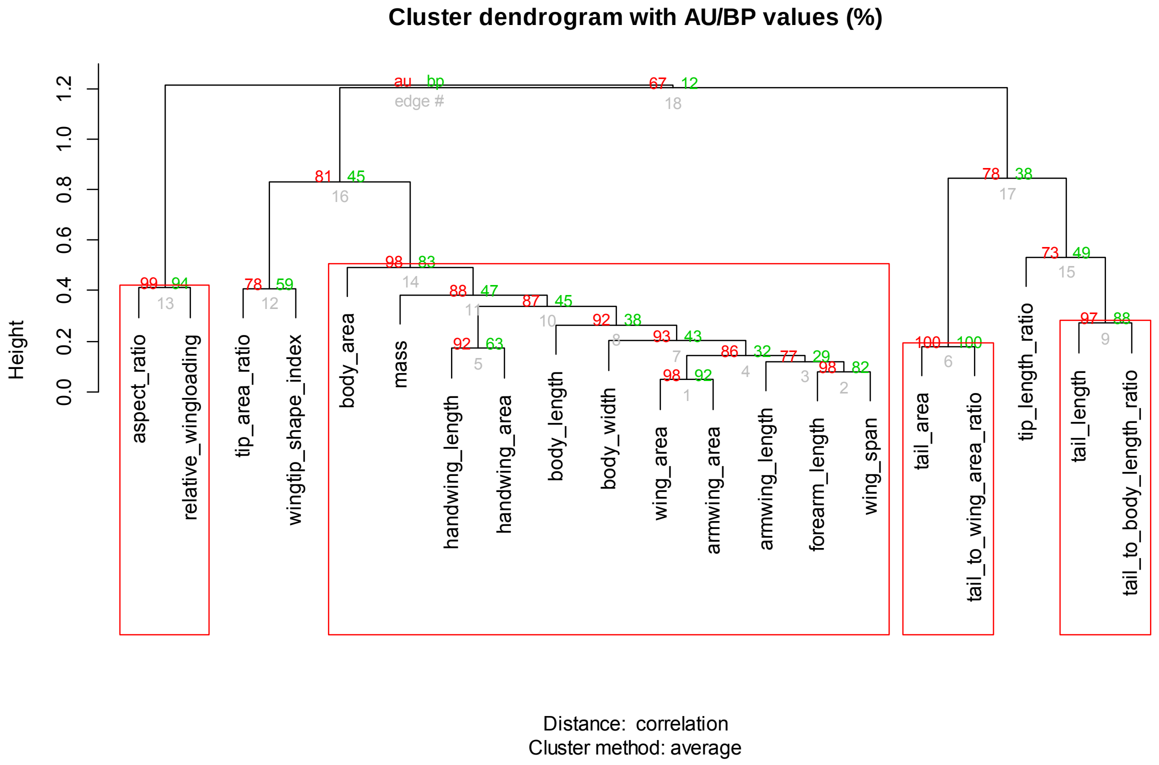

Figure 3.3: Hierarchical clustering of the 20 morphometric measurements, ratios and indices. The values presented at branches are approximate unbiased *p*-values (AU, left), bootstrap probability values (BP, right) and cluster labels (bottom). Clusters showing an AU ≥ 95 are indicated by rectangles. The first left rectangle corresponds to a cluster labelled 13, with an AU = 99 and BP = 94.

Differences in manoeuvrability between the species

The narrower the selected spacing, the greater the difference in the performance of the two species (Fig. 3.4). Therefore, we included the spacing as a variable in all of our GLMMs. Significantly lower numbers of unintended landings (effect between obstacle spacing and unintended landings, parameter estimate (Est.) ± standard error (SE) = -0.08 ± 0.00, *z* = -10.19, *p* < 0.001; effect between species and unintended landings Est. ± SE = -0.87 ± 0.27, *z* = -3.19, *p* =

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[1] Supplementary video S 1 is available on the journal website (http://www.nrcresearchpress.com/doi/abs/10.1139/cjz-2014-0104#.VHSVNsmE-cY).

0.001) and obstacle touches (spacing Est. ± SE = -0.05 ± 0·00, z = -13.40, p < 0.001; species Est. ± SE = -0.17 ± 0.08, z = -2.21, p = 0.027) were recorded for *M. blythii* compared with *M. myotis*. We found no effect regarding repeated measures for a given spacing, either for the obstacle touches (Est. ± SE = -0.04 ± 0.04, z = 0.96, p = 0.340) or for the unintended landings (Est. ± SE = 0.02 ± 0.07, z = 0.23, p = 0.816).

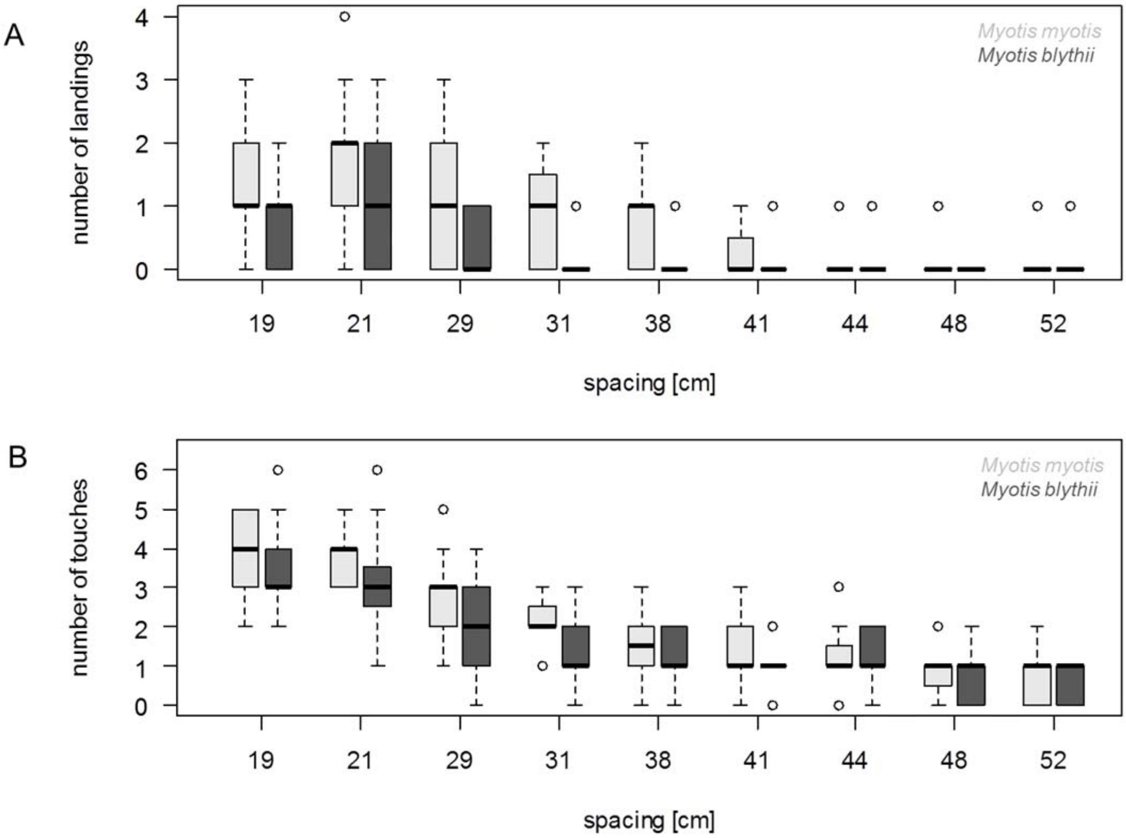


Figure 3.4: Performance in the obstacle course. The boxplot in 4 A shows the number of unintended landings per tested spacing and species (*Myotis myotis*, Borkhausen 1797, is indicated with light grey and *M. blythii,* Tomes 1857, with dark grey). The lower boxplot, in 4 B, shows the number of touches per tested spacing and species. The horizontal black bars are the medians; the grey boxes represent the interquartile ranges; the whiskers below and above represent the minimum and maximum values; and the circles represent outliers. The medians sometimes reach the end of the boxes because the dataset consists only of integer numbers.

Incorporating morphometric variables into the basic GLMM

When we tested all of the obtained morphological variables to determine their influence on the number of unintended landings each time in the same basic model, only two morphological variables exhibited significant corrected p-values:

the tail-to-body length ratio, with a corrected *p*-value of *p* <0.001, and body length, with *p* = 0.013 (Table 3.4). The model with the second lowest BIC included only the tail length and showed a lower BIC value compared with the model including body length, but in this case, the *p*-value for the tail length alone was not significant. The variable body length showed the only significant corrected *p*-value related to the overall size differences between the two tested species, although it was found to be correlated with other overall size-related variables, such as body width, wing span and wing area (cluster 14, Fig. 3.3).

For the response variable touches, none of the models exhibited significant variables (Table S 3.3).

Table 3.4: Basic generalized linear mixed effects models (GLMM) with response variable unintended landings and one added morphological variable (including body mass) for the greater and lesser mouse-eared bat (*Myotis myotis,* Borkhausen 1797 and *M. blythii,* Tomes 1857).

Model	BIC	Variable 2	Estimate	SE	*z*	*p*
L1	234.9	Tail-to-body length ratio	-6.71	1.54	-4.36	< 0.001
L2	244.4	Tail length	-82.56	65.16	-1.27	1.0 (ns)
L3	258.4	Body length	0.43	0.13	3.41	0.013
L4	262.7	Forearm length	0.34	0.16	2.19	0.574 (ns)
L5	263.6	Wing-tip shape index	0.32	0.17	1.89	1.0 (ns)
L6	263.6	Arm-wing length	0.31	0.16	1.93	1.0 (ns)
L7	263.7	Body width	0.31	0.16	1.94	1.0 (ns)
L8	263.9	Wing span	0.31	0.17	1.83	1.0 (ns)
L9	264.3	Hand-wing length	0.29	0.17	1.68	1.0 (ns)
L10+	265 - 267	All other morphological variables				1.0 (ns)

BIC ist the Bayesian information criterion. Variable 1 is the spacing that was always included in the models (spacing ± SE = -0.08 ±0.00, *z* = -10.19, *p*<0.001). The *p*-values of the morphological variables were corrected for multiple testing with a Bonferroni correction factor.

Forward selection modelling procedure with morphological variables

The forward selection procedure resulted in three models (with the lowest BIC values) that best accounted for the number of unintended landings (Table 3.5 and S 3.2). In addition to the spacing between obstacles and the random variable individuals, the models included the following variables: tail-to-body length ratio, body length, tail length, tip area ratio, relative wing loading and aspect ratio (Table 3.4). When the variables included in the three best models were compared with the cluster dendrogram, we did not find any highly correlated variables within each of the models. Only the second and third best models differed in presenting highly correlated variables relative to wing loading and the aspect ratio. The best model included only one additional variable, the tail-to-body length ratio. All variables exhibited significant *p*-values ranging from 0.005 to < 0.001 (Table 3.5). For the response variable touches, there was no best model with significant variables (Table S 3.3).

Table 3.5: Forward selection results show three best models with response variable unintended landings in the greater and lesser mouse-eared bat (*Myotis myotis,* Borkhausen 1797 and *M. blythii,* Tomes 1857).

BIC	Variable	Estimate	SE	*z*	*p*
Best model 1					
234.9	tail-to-body length ratio	-6.71	1.54	-4.36	< 0.001
Best model 2					
236.0	tail length	-93.4	28.03	-3.33	< 0.001
	body length	0.69	0.12	5.72	< 0.001
	tip area ratio	0.39	0.10	3.81	< 0.001
	relative wing loading	0.41	0.13	3.04	0.002
Best model 3					
237.7	tail length	-99.47	29.71	-3.35	< 0.001
	body length	0.51	0.09	5.85	< 0.001
	tip area ratio	0.30	0.09	3.30	< 0.001
	aspect ratio	0.25	0.09	2.81	0.005

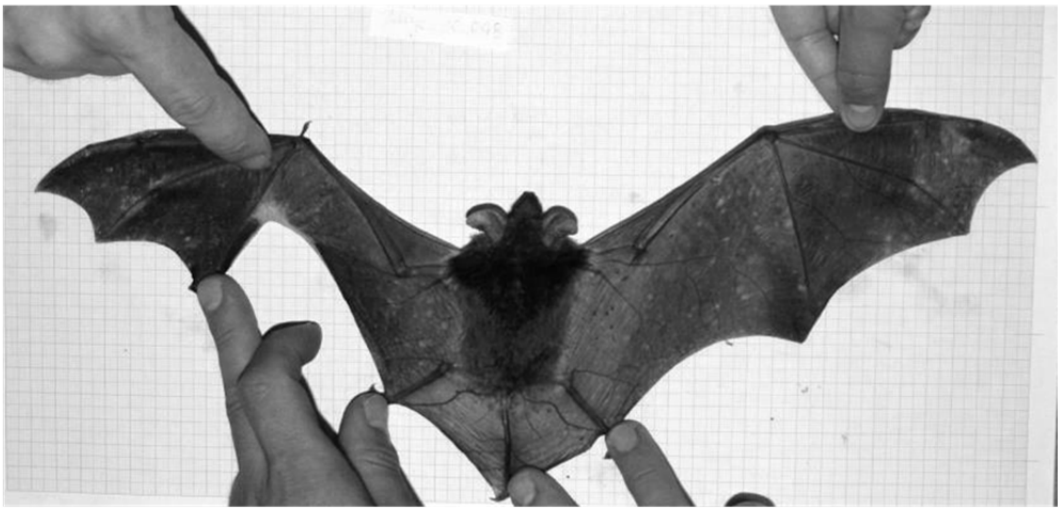
The variable spacing (spacing ± SE = -0.08 ±0.00, z = -10.19, p<0.001) was additionally included in the 3 best models. The *p*-value of model 1 was corrected for multiple testing with Bonferroni correction factor.

Comparison of individuals with injured wings

The injuries of both injured individuals were clearly visible (Fig. 3.5). The injured *M. myotis* individual showed a longer wing span, of 43.7 cm, compared with the other tested *M. myotis* individuals, with intact wing membranes. However, the mass of the injured *M. myotis* on the first night after capture was 25.0 g, which was slightly lower than the mass of most of the unharmed *M. myotis* (Table 3.2). All of the wing measurements for the left side of the injured *M. myotis* exhibited lower values compared with the right side. In the case of the arm-wing area, nearly 40 % of the area was missing on the left side compared with the right wing (Table S 3.2).

The wing span of the injured *M. blythii* was 37.2 cm, slightly lower than in most of the other tested individuals from the same species. The injured *M. blythii* was also lighter, showing a mass of 20.0 g, compared with all of the unharmed tested *M. blythii* (Table 3.2). Due to holes in the wing membrane of the injured *M. blythii*, 7.6 % of its left and 1.4 % of its right hand-wing area was missing (Fig. 3.5, table S 3.4). In both injured individuals, the aspect ratio (*M. myotis*: 8.38 and *M. blythii*: 7.32) and the relative wing loading (*M. myotis*: 36.80 N/m² and *M. blythii*: 38.21 N/m²) were greater than in the individuals with intact wing membranes.

A **Myotis myotis**



B **Myotis blythii**

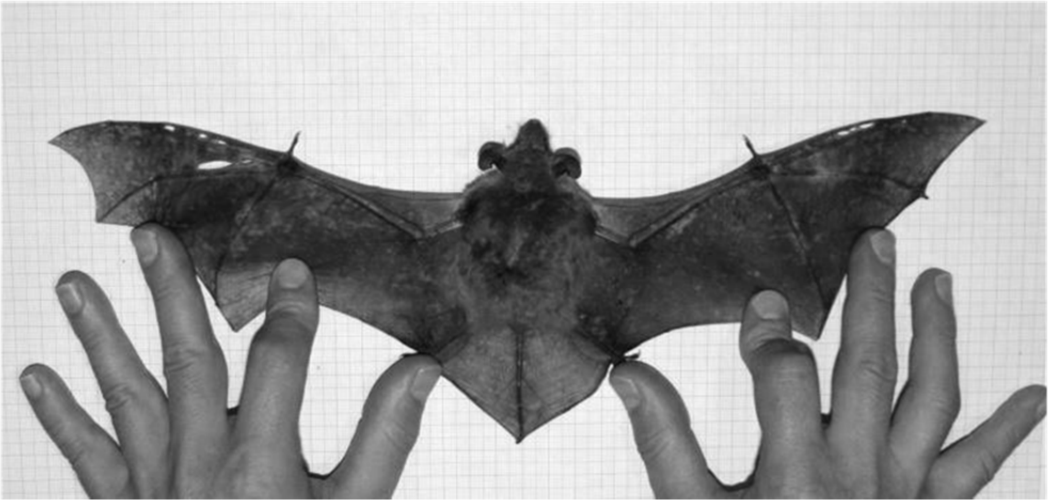


Figure 3.5: Wing pictures for one injured greater mouse-eared bat and one injured lesser mouse-eared bat (*Myotis myotis*, Borkhausen 1797, and *M. blythii*, Tomes 1857, respectively).

Both injured bats were successfully tested in the obstacle course. The injured *M. myotis* completed 23 valid trials (see for example video S 1), and the narrowest spacing that it could negotiate without touching any obstacle was 48 cm. The injured *M. blythii* completed 27 trials, and the narrowest spacing that it could negotiate without touching any obstacle was 31 cm.

DISCUSSION

Morphology

The differences observed between the two sibling species for most of the wing and body measurements (smaller values for *M. blythii* compared with *M. myotis*) were consistent with our assumption that the overall difference in size is the main factor that accounts for their differing manoeuvrability. However, not all of the morphometric measurements were significantly smaller for *M. blythii.* In these cases, the species showed deviation from simple overall size differences due to differences in shape (Table 3.1). We found significant differences in wing shape with regard to the tip length ratio and, more importantly, in the wing-tip shape index, independent of size. The wing-tip shape index was lower for *M. blythii* compared with *M. myotis.* A lower wing-tip shape index indicates a more pointed wing-tip and is known to enhance roll agility, which can increase manoeuvrability (through improved agility) (Norberg and Rayner, 1987). The more pointed wing-tips of *M. blythii* would therefore be favourable for performing quick movements around bushes and other vegetation. Conversely, the more rounded wing-tip of *M. myotis* can be an advantage when hovering (Norberg and Rayner, 1987), for example, when flying over the ground searching for prey (Russo et al., 2007, Dietz et al., 2009).

Unfortunately, there is no available index to test for differences in tail-tip shape, such as those observed for the wing. The longer tail of *M. blythii* relative to its size and the not significantly different tail-to-wing area ratio might indicate a more pointed tail compared with that recorded for *M. myotis*. Another possible explanation could be that the tail area measurements were not sufficiently accurate to reveal significant differences between the tail area ratios. While obtaining the wing pictures, we had difficulty in fully extending the tail membrane. We therefore suppose that, at least in some cases, the tail membrane area was underestimated. The longer tail of *M. blythii* and the possibly larger tail membrane in relation to its size might represent adaptations to its hunting strategy. In contrast to *M. myotis*, *M. blythii* appears to be better adapted to catching prey from vegetation such as grass stalks during flight (Arlettaz, 1996, 1999; Rainho et al., 2010), in which case a larger tail membrane could be advantageous. A longer tail and a larger tail membrane are thought to increase manoeuvrability and agility and can improve the likelihood of capturing insects at slower flight speeds (Norberg, 1994; Dudley, 2002; Gardiner et al., 2011*a*; Gardiner et al., 2011*b*; Adams et al., 2012).

Body area measurements showed no significant differences between the studied species, whereas the body length and body width were clearly significantly different. Overall, the body shape of *M. blythii* was more compact (body width was not reduced as much in a caudal direction) than that of *M. myotis,* as can be seen, for example, in the direct comparison of the body shapes of the two injured bats (Fig. 3.5).

The differing shapes of the wing-tip, body and especially the tail of *M. myotis* and *M. blythii* might be a consequence of character displacement (a process caused by competitive interactions among organisms that leads to divergent trait evolution) (Pfennig and Pfennig, 2010). Further measurements conducted in individuals from the two species from allopatric populations might help in validating this assumption.

Differences in manoeuvrability between species

The obstacle course experiment confirmed our hypothesis that *M. blythii* demonstrates greater manoeuvrability compared with its sister species *M. myotis.* The species differences were more significant for unintended landings than for touching the obstacles, but both results were well in line with each other. The weaker significance observed for the touches might have occurred because touching an obstacle is less harmful (and happens in nature as well), while landing on the ground can be dangerous and is energetically much more demanding (Davis, 1968; Voigt and Lewanzik, 2012; Lima and O'Keefe, 2013). Another possible explanation is that due to their different foraging behaviours, *M. myotis* lands on the ground more often than *M. blythii*. However, in this case, we would have expected to record many more invalid trials among the *M. myotis* individuals, including landing in front of the obstacles without obstacle touches or crawling through the course. Instead, we were only obliged to exclude a few trials, by one individual of *M. myotis* (2 trials) and two individuals of *M. blythii* (a total of 5 trials) due to repeated crawling or landing on purpose (without any touches). Furthermore, it is important to mention that we did not record how forcefully the bats touched the obstacles. A light touch with the wing-tip was counted in the same way as a strong touch with the inner wing area (arm-wing area) or even with the body. Strong touches could change the flight path more dramatically than a light touch. In most cases of unintended landings, one or several strong obstacle touches occurred just beforehand and forced the bat to land on the ground. Therefore, we consider the results obtained for unintended landings to be more powerful proof of manoeuvrability than the results for touches.

Incorporating morphometric variables into the basic GLMM

Of the two variables included in the models showing significant corrected *p*-values, body length seemed to play a major role because it was included in the tail-to-body length ratio measurement as well. At first glimpse, it might appear that the tail length only reached significance because it was related to the body length. On the other hand, one should take into account the fact that body length is well in line with the species-specific overall size differences, whereas this is not the case for tail length. Therefore, in the model, tail length must be considered in relation to overall size to show a significant difference in performance. We could still have obtained a significant *p*-value for the relative tail length if we had generated the ratio with a different variable representing overall size differences, such as wing span or forearm length. However, the obtained results also showed that the morphometric variables that contribute to overall size differences (cluster 14, Fig. 3.3) do not have the same importance for the manoeuvrability performance with regard to unintended landings. Contrary to our assumption, no significant *p*-value was found for the wing span. This result confirms findings reported by Gardiner et al. (2011*a*). We therefore concluded that in our experiments, wing span did not significantly affect the differences in the manoeuvrability performance of *M. myotis* and *M. blythii*.

Forward selection modelling procedure with morphological variables

The most important morphometric variables affecting unintended landings were body length, tail length and especially the newly defined tail-to-body length ratio (best model), which proved to be a highly significant morphological ratio differentiating the two species. Body length was the only variable that belonged to cluster 14 in our hierarchical clustering dendrogram describing the overall size differences between the two tested species. Interestingly, body length, tail length and the tail-to-body length ratio refer to the sagittal plane of the bat, and not, as initially assumed for the wing span, to the transverse plane. Differences in the sagittal plane might have a greater influence because they are closer to the centre of mass (Iriarte-Diaz and Swartz, 2008). We therefore concluded that the closer the impact was to the centre of mass of an obstacle, the higher the risk of an unintended landing. Furthermore, if we assume that the body length can only be slightly adjusted during flight, in contrast to the position of the wing, we suggest that during tight, quick turns close to obstacles, a greater body length would be unfavourable due to causing a reduced yaw ability and therefore a higher risk of obstacle touches or unintended landings on the ground (Swartz et al., 2012). Additionally, the longer tail of *M. blythii* appears to enhance the speed of body rotation and, thus, increases torsional agility, thus resulting in a lower risk of obstacle touches or unintended landings on the ground (Dudley, 2002).

Additional variables in the best models with the response variable unintended landings were located in the frontal plane. The tip area ratio was included in the best models. Interestingly, neither the tip length area nor wing-tip shape index appeared in the best models, although in our morphometric measurements, we found that these variables were significantly different between the two species. It appears that the tip area ratio measurement is more important for manoeuvrability in this case, independent of the shape of the wing tip. The tip area ratios for *M. myotis* and *M. blythii* (0.64 and 0.63, respectively) showed an intermediate value compared with other bat species, which is considered to be conducive to good manoeuvrability (Norberg and Rayner, 1987). Most of the wing mass is located in the arm-wing area, which is a component of the tip area ratio and plays an important role in slow flight as well as in maintaining stability during flight (Norberg and Rayner, 1987). Because the arm-wing area is closer to the body than the hand-wing area, the obstacle touches within the former area were stronger in most cases and were more likely to lead to landings on the ground than obstacle touches within the hand-wing area region (pers. observation). The relative wing loading and aspect ratio, which were also included in the three best models, were found to be highly correlated in our dendrogram. Therefore, these two models were interchangeable. The low relative wing loading and aspect ratio observed in both of the tested species enables slow flight, which should give a bat more time to manoeuvre around obstacles without touching them, or to adjust its flight path after a touch (Norberg and Rayner, 1987; Norberg, 1994).

The reason that we did not obtain significant variables for the response variable touches was most likely because this response variable did not differentiate sufficiently between the two species to allow investigation of the effect of the morphological variables. It might also be that factors other than the measured morphological variables, such as differences in behavioural flexibility (Clarin et al., 2013) or the flight speed while negotiating the obstacle course, played a role. On the other hand, it could be that the tested bats took light touches on the flexible obstacles into account after recognising that they were harmless.

Comparison of individuals with injured wings

The wing injuries of both injured individuals led to reduced wing membrane areas. In the injured *M. myotis* individual, the injury was particularly severe, as a large area of the left arm-wing membrane was missing. During flight, the asymmetrical wing areas may have generated differing lift and thrust forces on the two wings, which would likely have made it difficult for the bat to achieve straight or manoeuvred flight (Muijres et al., 2008; Song et al., 2008; Voigt, 2013). The injuries observed on the injured *M. blythii* individual were of a different kind, as less of its wing area was missing, and the injuries were spread over both hand-wing areas. Therefore, we would assume that achieving straight or manoeuvred flight should not have caused difficulties as severe as those for the injured *M. myotis.*

Consistent with Voigt (2013), who also studied the manoeuvres of naturally injured bats, our injured bats exhibited a low or lower initial mass as well as higher aspect ratios and relative wing loadings than most of the intact individuals of the same species. However, in our experiments, both injured bats performed well in the obstacle course, and we did not find differences in manoeuvrability compared with the tested healthy conspecifics. The injured bats successfully completed as many trials as the bats with intact wing membranes. This finding stands in contrast to the observations of Voigt (2013), who assumed that bats with wing injuries would avoid flight manoeuvres.

Conclusions

Ultimately, variations in overall size cannot account for the differences in the manoeuvrability of the two closely related species examined in this work. The morphological variables that contribute to variations in overall size were found to be of differing importance in relation to the manoeuvrability performance of the two tested species. The only variable that was related to overall size in the best models with significant variables was body length. These species show differences in several wing and body regions with respect to the overall trend in size (e.g., the smaller size of *M. blythii*), mainly regarding wing-tip shape and tail length. *M. blythii* performed better in the obstacle course and is therefore considered to be more manoeuvrable. Although differences in wing-tip shape exist, the most important morphological variables resulting in differing manoeuvrability in the two species were observed to be the tail-to-body length ratio and tail length, both of which are located in the sagittal plane. The tail of *M. blythii*, which is enlarged relative to its size, is therefore not only linked to its prey capture behaviour but also has an important influence on manoeuvrability. The results obtained from the two tested injured bats support the conclusion that tail morphology is more important than wing morphology in determining a bat’s manoeuvrability, as the tails of these bats were undamaged. We conclude that the morphometric differences in the overall size of the two tested species, and, more importantly, in their tail dimensions, constitute their main adaptations to different foraging and feeding strategies.

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SUPPLEMENTARY MATERIAL

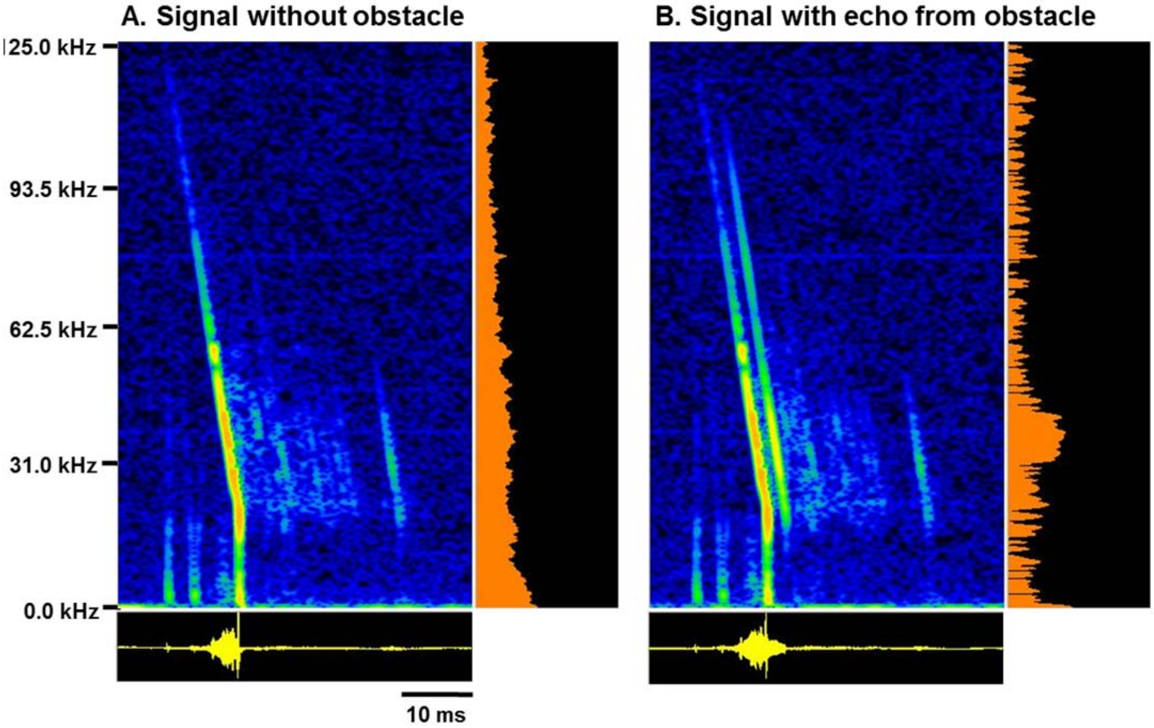

Figure S 3.1: Ensonification of an obstacle. Spectrogram with time signal below and averaged power spectrum to the right shows a recording of the played back signal without obstacle (A) and with obstacle (B). In B a distinct echo from the obstacle can be seen.

Table S 3.1. Definitions of ratios and indices used for comparison of relative size and shape differences in the studied greater (*Myotis myotis*) and lesser (*Myotis blythii*) mouse-eared bats.

Name	Symbol	Equation	Reference
Aspect ratio	AR	$\mathrm{AR} = L_{ws}^2 / S$	Norberg 1994
Relative wing loading	RWL	$\mathrm{RWL} = \mathrm{mass}{*}g / S{*}\mathrm{mass}^{1/3}$	Norberg 1994
Tip length ratio	T_l	$T_l = L_{hw} / L_{aw}$	Norberg & Rayner 1987
Tip area ratio	T_S	$T_S = S_{hw} / S_{aw}$	Norberg & Rayner 1987
Wing-tip shape index	I	$I = T_S / (T_l - T_S)$	Norberg & Rayner 1987
Tail-to-body length ratio	TB_l	$\mathrm{TB}_l = L_t / L_{bl}$	own definition
Tail-to-wing area ratio	TS_S	$\mathrm{TS}_S = S_t / (2\,S_{hw} + 2\,S_{aw} + S_b)$	own definition

Equations for the different ratios and indices are shown with reference to the respective authors. For relative wing loading, the mass was measured before the experiment started and *g* stands for the surface gravity (9.80665 $\mathrm{m/s^2}$).

Table S 3.2. All models tested during forward selection process with "unintended landings" as response variable

BIC	# var.	model	formula	comment
261	1	L0	glmer(landings~dist+(1|ID) , data=wm, family=poisson)	basic model
235	2	L1	glmer(landings~dist+tail-to-body length ratio+(1|ID) , data=wm, family=poisson)	Best model, $p < 0.001$
244	2	L2	glmer(landings~dist+tail length+(1|ID) , data=wm, family=poisson)	$p = 1.0$
258	2	L3	glmer(landings~dist+body length+(1|ID) , data=wm, family=poisson)	$p = 0.013$
263	2	L4	glmer(landings~dist+forearm length+(1|ID) , data=wm, family=poisson)	$p = 0.574$
264	2	L5	glmer(landings~dist+wing span+(1|ID) , data=wm, family=poisson)	$p = 1.0$
264	2	L6	glmer(landings~dist+hand-wing length+(1|ID) , data=wm, family=poisson)	$p = 1.0$
264	2	L7	glmer(landings~dist+arm-wing length+(1|ID) , data=wm, family=poisson)	$p = 1.0$
264	2	L8	glmer(landings~dist+wing-tip shape index+(1|ID) , data=wm, family=poisson)	$p = 1.0$
264	2	L9	glmer(landings~dist+body width+(1|ID) , data=wm, family=poisson)	$p = 1.0$
265 - 267	2	L10+	glmer(landings~dist**+other variables**+(1|ID) , data=wm, family=poisson)	every added variable $p = 1.0$
240	3	L15	glmer(landings~dist+body length+tail length+(1|ID) , data=wm, family=poisson)	
240	3	L13	glmer(landings~dist+tail length+body length+(1|ID) , data=wm, family=poisson)	
235 - 241	3	L11+	glmer(landings~dist+tail-to-body length ratio**+other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
243 - 250	3	L12+	glmer(landings~dist+tail length**+other variables***+(1|ID) , data=wm, family=poisson)	*except body length
261 - 264	3	L14+	glmer(landings~dist+body length**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail length
263 - 270	3	L16+	glmer(landings~dist+wing span**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (BIC 241) & tail length (BIC 246)
264 - 269	3	L19+	glmer(landings~dist+wing-tip shape index**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (239) & tail length (246)
264 - 270	3	L22+	glmer(landings~dist+hand-wing length**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (BIC 241) & tail length (BIC 248)
264 - 270	3	L25+	glmer(landings~dist+arm-wing length**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (BIC 241) , tail length (BIC 247)
264 - 271	3	L28+	glmer(landings~dist+tip area ratio**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (BIC 235), tail length (BIC 247) & body length (BIC 262)
264 - 269	3	L32+	glmer(landings~dist+forearm length**+other variables***+(1|ID) , data=wm, family=poisson)	*except tail to body length ratio (BIC 241), tail length (BIC 246)

234 - 245	4	L35+	glmer(landings~dist+tail-to-body length ratio+arm-wing area+**other variable**s+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
234 - 246	4	L36+	glmer(landings~dist+tail-to-body length ratio+arm-wing length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
237 - 246	4	L37+	glmer(landings~dist+tail-to-body length ratio+forearm length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
238 - 246	4	L38+	glmer(landings~dist+tail-to-body length ratio+wing span+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
239 - 246	4	L39+	glmer(landings~dist+tail-to-body length ratio+hand-wing length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
239 - 246	4	L40+	glmer(landings~dist+tail length+body length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
240 - 246	4	L41+	glmer(landings~dist+body length+tail length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
244 - 252	4	L42+	glmer(landings~dist+tail length+forearm length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
245 - 254	4	L43+	glmer(landings~dist+tail length+arm-wing length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
245 - 252	4	L44+	glmer(landings~dist+tail length+wing span+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
245 - 253	4	L45+	glmer(landings~dist+tail length+hand-wing length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
257 - 270	4	L46+	glmer(landings~dist+body length+forearm length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail length (BIC 246)
263 - 270	4	L47+	glmer(landings~dist+body length+arm-wing length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio & tail length (BIC 245 & 245)
264 - 270	4	L48+	glmer(landings~dist+body length+wing span+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio & tail length (BIC 245 & 246)
266 - 270	4	L49+	glmer(landings~dist+body length+hand-wing length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio & tail length (BIC 245 & 246)
236	5	L51	glmer(landings~dist+tail length+body length+tip area ratio+relative wingloading+(1|ID) , data=wm, family=poisson)	2nd best model
238	5	L52	glmer(landings~dist+tail length+body length+tip area ratio+aspect ratio+(1|ID) , data=wm, family=poisson)	3rd best model
238	5	L53	glmer(landings~dist+tail length+body length+tip area ratio+wing area+(1|ID) , data=wm, family=poisson)	
239 - 245	5	L50+	glmer(landings~dist+tail length+body length+tip area ratio+**other variables***+(1|ID) , data=wm, family=poisson)	*except aspect ratio, wing area & relative wingloading
237 - 244	6	L54+	glmer(landings~dist+tail length+body length+tip area ratio+wing area+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
239 - 242	6	L55+	glmer(landings~dist+tail length+body length+tip area ratio+relative wingloading+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
240 - 243	6	L56+	glmer(landings~dist+tail length+body length+tip area ratio+aspect ratio+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better

For the forward selection process, we added all length and area measurements (Table 3.2), as well as all indices and ratios (Table 3.3) and mass, as explanatory variables to the basic model L0. The basic model consists of the variable space between the obstacles (dist) and the individual as a random factor (1|ID). The green colour indicates the three best models and the red colour indicates models that were excluded because their BIC value was not at least two units better than the BIC values of simpler models with one or several of the same variables. The *p*--values for the univariate GLMMs were adjusted for multiple testing with a Bonferroni-correction factor.

Table S 3.3. All models tested during forward selection process with "touches" as a response variable

BIC	# var.	model	formula	comment
183	1	T0	glmer(touches~dist+(1|ID) , data=wm, family=poisson)	basic model
176	2	T1	glmer(touches~dist+tail length+(1|ID) , data=wm, family=poisson)	p = 1.0
177	2	T2	glmer(touches~dist+tail-to-body length ratio+(1|ID) , data=wm, family=poisson)	p = 1.0
186	2	T3	glmer(touches~dist+forearm length+(1|ID) , data=wm, family=poisson)	p = 1.0
187	2	T4	glmer(touches~dist+arm-wing length+(1|ID) , data=wm, family=poisson)	p = 1.0
187	2	T5	glmer(touches~dist+tip length ratio+(1|ID) , data=wm, family=poisson)	p = 1.0
188	2	T6	glmer(touches~dist+wing span+(1|ID) , data=wm, family=poisson)	p = 1.0
188	2	T7	glmer(touches~dist+hand-wing length+(1|ID) , data=wm, family=poisson)	p = 1.0
188	2	T8	glmer(touches~dist+arm-wing area+(1|ID) , data=wm, family=poisson)	p = 1.0
188	2	T9	glmer(touches~dist+wing-tip shape index+(1|ID) , data=wm, family=poisson)	p = 1.0
189 - 190	2	T10+	glmer(touches~dist+**other variables**+(1|ID) , data=wm, family=poisson)	every added variable p = 1.0
179 - 181	3	T11+	glmer(touches~dist+tail length+other variables+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
180 - 183	3	T12+	glmer(touches~dist+tail-to-body length ratio+other variables+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
188 - 192	3	T13+	glmer(touches~dist+forearm length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 182) & tail length (BIC 180)
188 - 194	3	T16+	glmer(touches~dist+wing span+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 183) & tail length (BIC 181)
188 - 194	3	T19+	glmer(touches~dist+arm-wing area+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 184) & tail length (BIC 181)
189 - 195	3	T22+	glmer(touches~dist+hand-wing length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 183) & tail length (BIC 181)
187 - 194	3	T25+	glmer(touches~dist+arm-wing length+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 183) & tail length (BIC 181)
189 - 194	3	T28+	glmer(touches~dist+tip length ratio+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 183) & tail length (BIC 180)
190 - 194	3	T31+	glmer(touches~dist+wing-tip shape index+**other variables***+(1|ID) , data=wm, family=poisson)	*except tail-to-body length ratio (BIC 184) & tail length (BIC 180)
183 - 185	4	T34+	glmer(touches~dist+tail length+aspect ratio+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
183 - 186	4	T35+	glmer(touches~dist+tail length+tip length ratio+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
181 - 188	4	T36+	glmer(touches~dist+tail-to-body length ratio+forearm length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
182 - 189	4	T37+	glmer(touches~dist+tail-to-body length ratio+tip length ratio+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
183 - 189	4	T38+	glmer(touches~dist+tail-to-body length ratio+arm-wing length+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better
185 - 188	4	T39+	glmer(touches~dist+tail-to-body length ratio+aspect ratio+**other variables**+(1|ID) , data=wm, family=poisson)	BIC not 2 units better

For the forward selection process, we added all length and area measurements (Table 2), as well as all indices and ratios (Table 3) and mass, as explanatory variables to the basic model T0. The basic model consists of the variables space between the obstacles (dist) and the individual as random factor (1|ID). The red colour indicates models that were excluded because their BIC value was not at least two units better than the BIC values of simpler models with one or several of the same variables. The *p*- values for the univariate GLMMs were adjusted for multiple testing with a Bonferroni-correction factor.

Table S 3.4. Length and wing area measurements of the injured greater (*Myotis myotis*) and lesser (*Myotis blythii*) mouse eared bat.

species	*N*	wing span, (cm)	hand-wing length (cm)		arm-wing length (cm)		hand-wing area (cm^2)		arm-wing area (cm^2)		wing area (cm^2)	area of holes (cm^2)		aspect ratio	relative wing loading (N/m^2)
			left	right	left	right	left	right	left	right		left	right		
M. myotis	1	43.7	7.1	8.0	7.6	8.4	33.9	45.6	**37.3**	61.7	227.9	-	-	8.38	36.80
M. blythii	1	37.2	8.7	8.6	6.5	6.2	**26.7**	**27.5**	43.2	43.5	189.1	2.2	0.6	7.32	38.21

Note: The measurements are shown for the left and right wing and the numbers in boldface type indicate membrane areas where the injuries were located.

4. Manoeuvrability in horseshoe bats

Niche differentiation in sympatric horseshoe bats (Rhinolophidae): the importance of obstacle avoidance and its morphological correlates

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ABSTRACT

Niche differentiation limits similarity among coexisting species. Evidence of resource partitioning between insectivorous bat species is typically inferred from differences in ecomorphological traits that influence detection and capture of insect prey, notably wing morphology and echolocation signal design. However, in some closely related species morphological differences can be small and their influence on ecological performance uncertain.

In Southeastern Europe, all five European horseshoe bat species (*Rhinolophus spp.)* occur sympatrically. They are presumed to compete for prey during periods of low insect abundance and are known to forage within or close to vegetation within woodlands. In this habitat, manoeuvrable flight is essential to avoid collisions with vegetation during prey pursuit.

We hypothesized that resource partitioning in these species is effected by differences in their morphology producing variation in the ability to manoeuvre within dense vegetation. We tested the ability of several individuals of each species to pass through obstacle courses simulating vegetation of different densities. The resulting manoeuvrability performances were then related to individual variation in wing, body and tail phenotype.

The five species differed mainly in their overall size measurements (all length and area measures that are proportional to each other), although there was overlap among the intermediate-sized species for some measurements (arm-wing area, tail length, tail area, body length and body area). When relating the manoeuvrability performance to morphometric measurements we found that differences in mass and body length played a major role in interspecific differences in manoeuvrability. Generally, smaller species performed better than the larger species in the obstacle experiment, with the exception of *Rhinolophus blasii*, which had a longer body length (relative to its size). Although *R. blasii* is generally the second smallest bat, it performed only as well or even poorer as the second largest bat *Rhinolophus mehelyi*. Measures that were directly related to the wing were found to have a less prominent role for the manoeuvrability performance of the horseshoe bats.

Although all five species are well-adapted to forage in dense vegetation and differences in manoeuvrability among them were small, differences in overall size, rather than wing morphology per se, appear to provide potential for ecological separation. However, mainly in the intermediate sized horseshoe bat species some morphometric variables deviated from the overall size trend (especially body length) and played a prominent role in flight performance, suggesting size-independent influences of shape on niche differentiation for species that are very similar in (overall) size.

INTRODUCTION

Niche partitioning has long been known as an important mechanism facilitating species coexistence (Schoener 1974; Chesson 2000; Leibold & McPeek 2006; Levine & HilleRisLambers 2009). In sympatric bat species, adaptations to different foraging niches are indicated by clear differences in echolocation (Neuweiler 1984; Schnitzler & Kalko 2001; Siemers & Schnitzler 2004), and size or shape of wings (Aldridge & Rautenbach 1987; Norberg & Rayner 1987; Norberg 1994; Stockwell 2001). However, in some closely related and ecologically similar species, morphological differences indicative of niche partitioning can be small (Saunders & Barclay 1992; Jacobs et al., 2007; Jacobs & Barclay 2009; Krueger et al. 2014; Schmieder, Zsebők & Siemers 2014) and their influence on ecological performance uncertain. Investigating how these similar species differ provides insights into the mechanisms that promote coexistence and specialization in foraging niches.

Vegetatively complex habitats, such as forests and forest edges, provide abundant insect resources (Price et al. 2011), but bats must be capable of slow, manoeuvrable flight to pursue prey while also avoiding collisions with the vegetation (Norberg 1994). Manoeuvrability can be defined as the ability to fly through limited space while avoiding vegetation or other obstacles and, in general, correlates negatively with wing loading (body mass x gravity / area of the wing) (Norberg & Rayner 1987). Bats with low wing loading typically have low body mass (Aldridge & Rautenbach 1987; Stockwell 2001; Sleep & Brigham 2003), and relatively large wing area, so manoeuvrable bats tend to have broad wings and low mass for a given size (Norberg 1994). Wingspans are short and wing tips rounded, and broad wings and a large tail membrane improve the ability to make rapid changes of direction, further enhancing manoeuvrability (Norberg 1994; Dudley 2002; Gardiner et al., 2011; Gardiner et al. 2011; Adams et al., 2012).

In Europe, five species of horseshoe bats (*Rhinolophus spp.*) frequently hunt within or close to vegetation. In Southeastern Europe (Bosnia-Herzegovina, Bulgaria, Greece, Macedonia, Montenegro, Serbia and Turkey) all five species occur sympatrically, yet the mechanisms promoting coexistence of such similar species are unknown. Although these species differ to some extent in diet and hunting strategies (Russo et al. 2005; Dietz, von Helversen & Nill 2009; Salsamendi et al. 2012), they can overlap in diet (Goiti et al., 2004), foraging behaviour (Jones & Rayner 1989; Ahmim & Moali 2013), foraging habitat (Bontadina et al., 2002; Russo et al., 2002; Aihartza et al. 2003; Reiter 2004; Goiti et al. 2008; Salsamendi et al. 2008) and echolocation (Russo et al. 2007; Schuchmann & Siemers 2010). Although shifts in hunting behaviour and diet have also been documented (Siemers & Ivanova 2004; Dietz et al., 2009; Flanders & Jones 2009; Dietz et al., 2013), competition for resources has been established for two of the five European horseshoe bat species (*Rhinolophus euryale* Blasius, 1853 and *R. mehelyi* Matschie, 1901) (Russo et al. 2014).

Other mechanisms that might promote resource partitioning of the European horseshoe bats are morphological differences among the species (Andreas et al. 2013). The five horseshoe bat species differ in overall size. Overall size is here defined as the geometrical similarity of the shape and weight including all measures within the shape (i.e., all measures that are proportional to each other; see wing picture in Fig. 4.1) of the different tested species. In this context one species shape can be obtained from the other species shape by uniformly scaling (enlarging or reducing), Shape differences can be recognized in case measures deviate from the overall size trend.

The smallest species *R. hipposideros* (Bechstein, 1800) (body mass 4-7g) and the largest species *R*. *ferrumequinum* (Schreber, 1774) (18-24g) can be easily differentiated by their size (Dietz et al., 2009). For the three intermediate species less distinct differences in size exist, however *R. mehelyi* is generally larger than *R. euryale*, and *R. euryale* is in most cases larger than *R. blasii*

(Peters, 1866) (Dietz et al., 2006; Salsamendi et al. 2012). The five species also differ slightly in wing and tail morphology (Dietz et al., 2006; Salsamendi et al. 2006).

Despite these suggestions and speculations that the morphological differences among the horseshoe bat species may facilitate niche differentiation, a clear link between morphology and flight performance has yet to be established. Here we compare external morphology with species' performance in an obstacle course designed to test manoeuvrability. Because manoeuvrability is generally related to wing loading and wing shape (Norberg & Rayner 1987), we predicted that differences in wing parameters (i.e., wing span, wing area) and body mass would play the main role in differences in manoeuvrability and that performance would reflect the overall size order of the bats. Therefore the smallest horseshoe bat, *R. hipposideros* should be the most manoeuvrable, since it has the shortest wing span and the lowest body mass which results in the lowest wing loading. As the three intermediate-sized species, *R. mehelyi*, *R. euryale* and *R. blasii* are very similar in mass and wing span we anticipated that any differences in performance would be attributable to wing shape divergence.

MATERIAL AND METHODS

Animals

We captured adult males of *R. hipposideros* (two individuals), *R. blasii* (seven), *R. euryale* (six), *R. mehelyi* (six) and *R. ferrumequinum* (six) with a harp trap (Faunatech, Victoria, Australia) or mist nets (Ecotone, Sopot, Poland) set at night at the entrance of caves in Bulgaria (districts: Ruse, Gabrovo, Lovech and Kardzhali) from May until September 2011. Capture, husbandry and behavioural studies were carried out under licenses from the responsible Bulgarian authorities (MOEWSofia and RIOSV-Ruse permit numbers 297/09.03.2011 and 205/29.05.2009). All bats were sexed, weighed (electronic scale, Kern, EMB 500-1, d = 0.1 g) and standard measures (wing span, body length, length of digit 5 and tail length) were taken with a dial caliper (Measy 2000 caliper, Switzerland, precision 0.1 mm) following Dietz and von Helversen (2004). We identified the five species according to Dietz et al. (2009). The bats were kept for up to 16 days at the nearby Tabachka Bat Research Station (Bulgaria) of the Sensory Ecology Group (Max-Planck Institute for Ornithology, Seewiesen, Germany). The bats were housed in flight tents (220 x 90 x 110 cm or 220 x 130 x 134 cm) at 20 °C ± 3 °C and 14:10 h light:dark photoperiod and released at or close to the site of capture after the completion of the experiments. We fed the bats during the experiments and if necessary we gave them additional mealworms (larvae of *Tenebrio molitor*, Coleoptera) after the experiments to maintain their body condition. Body mass was measured each day to monitor body condition.

Wing morphology

In addition to the standard measures, wing photographs were taken. We placed each individual with its ventral side firmly against the board of a copy stand (custom-made, Max-Planck Institute for Ornithology, Seewiesen, Germany) covered with graph paper and a transparent self-adhesive cover film. We carefully extended each wing, so that the humerus was at a 90° angle to the midline of the body. The wings and tail membrane were fixed with transparent adhesive tape (width 19 mm) to the board. We took several digital photographs of each bat with a digital camera (12 megapixel, DMC-TZ10, Panasonic, Osaka, Japan) that was attached to the copy stand (height 47 cm). After taking the photographs, we carefully removed the tape without injury to the bat. We chose the two best wing photographs (i.e., without blurriness in the picture, both wings were fixated symmetrically, the head was straight and pointed towards the plate and wing and tail membranes were fully extended) of each individual for analysis. From the selected wing photographs, we measured different lengths (hand-wing length, arm-wing length, upper arm and body width length) and areas (arm-wing area, hand-wing area, tail area, body area and wing area) (Fig. 4.1) with an image processing program (Adobe Photoshop, version 13.0.1, Adobe Systems, San Jose, USA). The measurements on the arm-wing and hand-wing were always taken from the right wing to keep consistency and because we anticipated that measures were similar across wings.

From the measurements of the wing photographs and the standard morphometric measures, we calculated aspect ratio (wing span^2 / wing area), relative wing loading (mass x gravity / wing area x mass^(1/3)), tip length ratio (hand-wing length / arm-wing length), tip area ratio (hand-wing area / arm-wing area), the wing-tip shape index (tip area ratio / (tip length ratio - tip area ratio)) (Norberg & Rayner 1987; Norberg 1994), tail-to-body length ratio (tail length / body length) and tail-to-wing area ratio (tail area / wing area - tail area) (Schmieder et al., 2014). We compared each measurement, ratio and index for species differences among the five horseshoe bat species and among the three intermediate species with a non-parametric Kruskal-Wallis test in R (version: 3.1.1, 2014-07-10) (RcoreTeam, 2014). The *p*-values were corrected for multiple testing with the function p.adjust in R and the Bonferroni correction. The 95% confidence intervals of the median values from the differing parameters were calculated using the bootstrapping method in R in the package boot (type: percentile) (Canty & Ripley 2013).

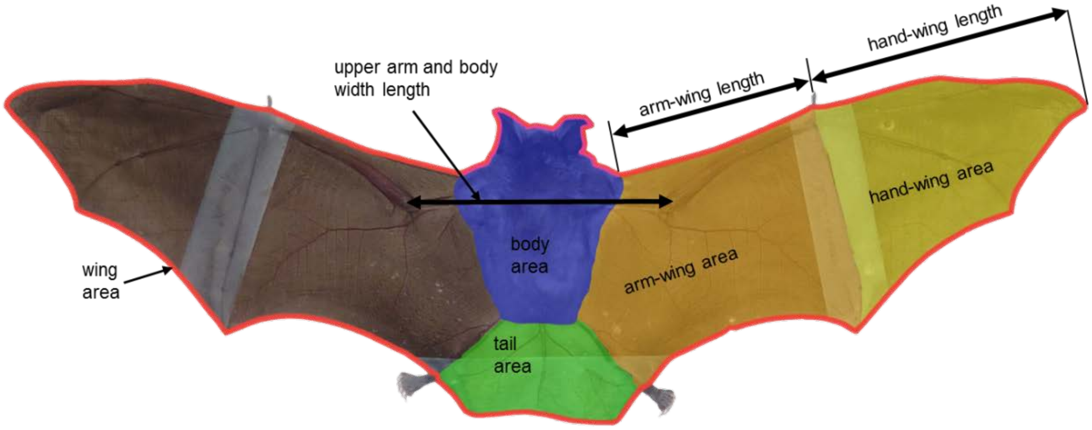

Figure 4.1: Wing of *Rhinolophus mehelyi* Borkhausen 1797 to illustrate morphometric measurements taken from photographs.

Manoeuvrability experiments

Flight performance of each species was assessed using an obstacle course of three rows of vertical plastic tubes (grey PVC, length approx. 2.30 m) set in the centre of a flight tunnel (length 8 m x breadth 1.5 m x height 2.40 m). The tubes had a diameter of 6 mm and a roughened surface and were clearly detectable by the tested bats (Schnitzler 1968; Sumer et al., 2009; Schmieder et al,. 2014).

The obstacle tubes were suspended with hooks from a wooden plate and fitted into holes in another wooden plate set on the ground. The wooden plates were attached to a wooden framework which we placed four metres away from the beginning of the flight tunnel (Fig. 4.2). The spacing between the obstacles was equal within and among the three rows. Each trial was monitored by the observer with a red headlamp light, and videos were recorded with 4 IR-cameras and Digi Protect (ABUS, Affing, Germany) surveillance software for later analysis. Infrared light was provided through a stroboscope (875 nm wavelength, flash rate 50 Hz, i.e., one flash per video half-frame, custom made, University of Tuebingen).

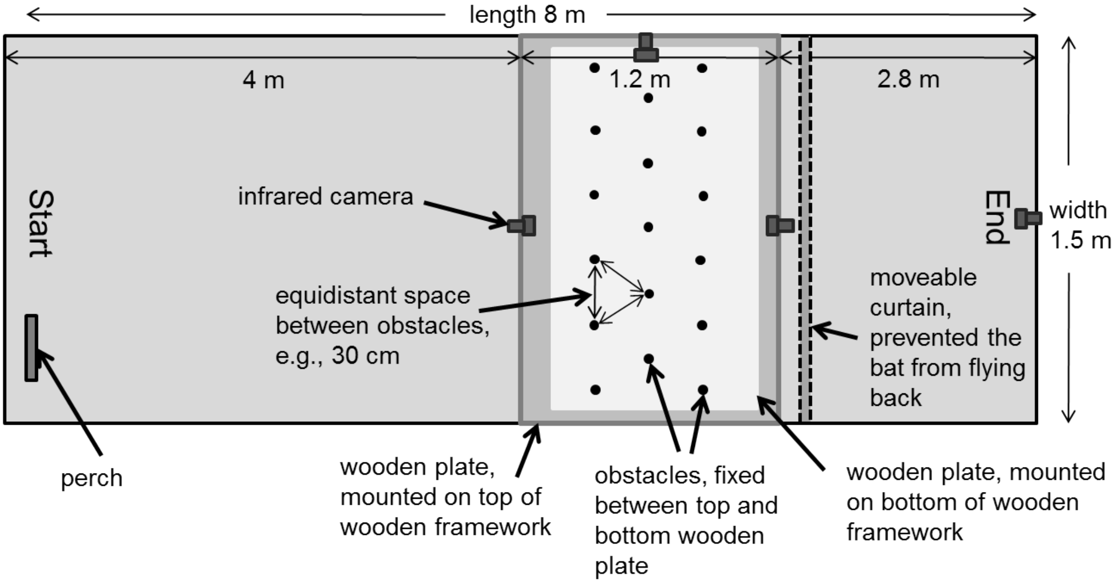

Figure 4.2: Flight tunnel - view from above. Sketch of the experimental set up (not exactly to scale), showing the flight tunnel and the wooden plates in the middle, where the obstacles were positioned. The bats flew from the Start position, through the obstacle course, to the End, where they received a reward.

Over the course of the experiment, each individual bat was presented with the same eight obstacle spacing distances (45 cm, 40 cm, 35 cm, 30 cm, 25 cm, 20 cm, 15 cm and 10 cm). The obstacle arrangements were presented beginning with the largest and ending with the smallest spacing. Each time all obstacle spacing distances were presented to an individual a so called "round" was completed. Each obstacle spacing was presented consecutively six times ("trials") during one round. When all six trials for a given spacing were presented to an individual one "presentation" was finished. After the smallest possible obstacle spacing was presented, a new round started with the widest obstacle spacing, until three rounds of presentations for each spacing were completed.

By presenting the same obstacle spacing consecutively, we attempted to test the bats' maximal manoeuvrability performance. We predicted the bats would get used to the obstacle positions by spatial learning (Barchi et al., 2013) and would improve their performance with time. This would resemble flight behaviour in known, cluttered habitat, such as vegetation with trees and bushes. The first presentation of an obstacle spacing, should somewhat resemble flight in unknown habitat because the bats were not used (no spatial learning effect) to the arrangement.

The experiments began one or two nights after capture. For the first three trials of an experimental night, we let each bat fly through the tunnel without obstacles. At the beginning of each trial, the bat was placed in a hanging position on a perch. It had three minutes' time to start flying on its own. If it did not start to fly, the perch was turned gently prompting the bat to fly. The bat was allowed three minutes to fly through the obstacle course to the end of the flight tunnel. If the bat failed to negotiate the course, instead, remaining near the obstacle course (e.g., by landing on the wall or ceiling in front of the obstacle course), it was taken and re-released at the beginning of the tunnel. If a bat refused to fly through the course for more than three minutes, the trial was stopped and the bat was again placed hanging on its perch, which was covered with a small cage (carton, height 27 cm x breadth 27 cm x depth 18 cm) for one minute to prevent the bat from flying and to signal a clear break between the two trials to the bat. If a bat did not manage to complete two of the six consecutive trials successfully – i.e., flying through the course without landing – for a given presentation in a round, the presentation was not completed successfully and, for the safety of the bat, we decided that it reached a so called “welfare threshold” where no smaller spacing was presented and a new round was started. Each time a bat completed one flight through the obstacle course, it was rewarded with 1 - 2 mealworms (and water from a pipet if needed) at the end of the flight tunnel. After the feeding break of about one minute a new trial was started until the presentation was completed and a maximum of 12 mealworms were fed (more mealworms reduced bats' motivation to undertake the trials). No more than three presentations per bat were conducted per night with a break between presentations of at least one hour.

The experimental procedure had to be adjusted for the two *R. hipposideros* because the species is very fragile (Weinberger et al., 2009) and therefore, each individual was only kept in captivity for half a night. Neither of the two individuals was tested at the 30 cm and 40 cm spacing and the welfare threshold was reached if a bat failed any of the first three trials. The bats were not tame enough to be rewarded with mealworms, but were given longer resting times (6-10 minutes) at the end of the flight tunnel between trials.

Video recordings of the trials were then inspected, and trials scored (performance score, Table 4.1) for soft and hard touches of the obstacle tubes, unintended landings (landing on ground after touching one or several obstacles) as well as invalid trials and the duration of the time it took the bat to cross the course (transition time) were noted. Trials were excluded from the analyses if a bat was disturbed directly before crossing the course or if there were technical problems with the video recording.

Table 4.1: Scoring system

Points	Description
10	No touch
9	One touch – soft or not clear
8	One touch – hard
7	Two touches
6	Three or more touches
5	Touches & one unintended landing on ground
4	Touches & two or more unintended landings
3	Invalid trial: wall
2	Invalid trial: landing on purpose (no touch)
1	Refused to fly through, crawling, welfare threshold

Differences in manoeuvrability

We employed generalized linear mixed effects models (GLMMs) in R (RcoreTeam, 2014) using the cumulative link mixed model (clmm) function function in the package ordinal (Christensen 2014) to test for differences in manoeuvrability (i.e., differences in performance score) among the species, obstacle spacing and repeated measures (rounds and trials). We fitted 19 competing models with alternative fixed effects structures and ranked them using Akaike information criterion corrected for small-sample-size (AICc; Akaike 1974; Hurvich & Tsai 1989; Mazerolle 2014) to determine which model best describes our data set (Table S 4.1, Fig. S 4.1). We accounted for individual differences and pseudo-replication by using individual identity as a random factor.

Differences in transition time

We employed GLMMs in R (RcoreTeam, 2014) using the lmer function in the package lme4 (Bates, 2013) modelled with a normal error distribution to check for differences in transition time (time for the bats to cross the obstacle course) of species, obstacle spacing and repeated measures (rounds and trials). For this analysis, we took only transition time measures for completed trials (those with a scoring of six or higher). We fitted 27 competing models with alternative fixed effects structures and ranked them using the AICc to determine which best describes our data set (Table S 4.2). As above, we accounted for individual differences and pseudo-replication by using individual identity as a random factor in all our models.

Relationship between morphology and flight performance – with single morphometric variables

To determine whether morphometric variables explained the observed species-specific differences in performance, the morphometric measures and ratios (Table 4.2 and 4.3) for the individuals tested were used within the modelling process. The 20 morphometric measures and ratios were added to the model with lowest AICc value which did not include species identity or the interaction with round (Table S 4.1, model 4 = basic model). Each time, only one variable was added to this basic model (20 variables = 20 models). All measured and calculated morphometric variables were included in the modelling process. We retained correlated variables because correlation does not guarantee the same functional role in manoeuvrability. For the added morphometric variables in the differing models, the *p*-values were corrected for multiple testing with a Bonferroni correction factor. The morphometric variables, their AICc values and their *p*-values were directly compared and discussed regarding the influence, or lack thereof, in relation to the manoeuvrability performance (Table S 4.3 A). A model with an AICc value at least two units lower compared with other models was considered to be the better model. We then repeated the procedure with a reduced dataset that included only the three intermediate species (Table S 4.4 A).

Relationship between morphology and flight performance – with multiple morphometric variables

Subsequently, we tested whether there was a minimum adequate model (MAM) of morphometric variables that best explained differences in manoeuvrability. We made shortlists (final selection A - C, Table S 4.3 B) of the variables from the three best single-variable models (lowest AICc values, Table S 4.3 A) and added any remaining morphometric variables that were not highly correlated (≥│0.7│) with the shortlisted variables (Table S 4.5). We then used the function “dredge” of the R package MuMIn (Barton 2014) with the three models including all morphometric variables from the shortlists to generate preliminary best models (best model A - C) and to find amongst those the best final model or MAM with the lowest AICc value. We repeated the procedure with a reduced dataset, including only the three intermediate-sized species, *Rhinolophus blasii, R. euryale* and *R. mehelyi* (Table S 4.4 B and S 4.6).

RESULTS

Wing morphology

There was a significant effect of species on all twelve length and area measurements (Table 4.2). However, when analyses were restricted to the three intermediate species, no significant differences were found for arm-wing area, tail length, tail area, body length and body area. For the size-independent ratios, we found no significant effect of species on relative wing loading, tip length ratio, tip area ratio, wing-tip shape index and the tail-to-wing area ratio (Table 4.3). Species did affect aspect ratio and the tail-to-body length ratio, but the latter did not differ among the three intermediate species. The lowest aspect ratio was found for *R. hipposideros* and the highest for *R. ferrumequinum*. *R. hipposideros*, followed by *R. ferrumequinum,* had the highest values of tail-to-body length ratio (i.e., longest relative tail length) and *R. blasii* had the lowest values (shortest relative tail length).

Manoeuvrability experiments

Flight performances of all bats except two *R. blasii* were successfully tested by the obstacle course. The two *R. blasii* were excluded because it took too long to recapture them between trials. After incomplete trials (due to disturbance or technical problems) were excluded, we obtained 64 trials for the two *R. hipposideros*, 720 for the remaining five *R. blasii*, 863 for the six

R. euryale, 862 for the six *R. mehelyi* and 857 for the six *R. ferrumequinum* in total (including the trials that reached the welfare threshold).

The narrowest obstacle spacing a bat negotiated without touching any obstacle was 15 cm achieved by one *R. euryale* in the 3rd round (average minimum negotiated spacing for *R. euryale*: 20.0 cm, N = 6). However, in the first round, the best performance was from the two *R. hipposideros* (Fig. 4.3 and S 4.2). Both individuals managed to manoeuvre through an obstacle spacing of 20 cm without any touches in several trials. *R. euryale* performed worse in the first round than *R. blasii* and *R. mehelyi*. *R. blasii* (average minimum negotiated spacing: 23.0 cm, N = 5) performed similarly or worse than *R. mehelyi* for most obstacle spacings and differing rounds (average minimum negotiated spacing: 22.5 cm, N = 6) (Fig. S 4.2). The poorest performance was by *R. ferrumequinum* (average minimum negotiated spacing: 25.0 cm, N = 6).

Crawling on the ground was observed in the obstacle course at narrow spacing (15 - 25 cm) for *R. blasii* (2 individuals, 9 trials), *R. mehelyi* (1 individual, 1 trial) and *R. ferrumequinum* (1 individual, 1 trial). Furthermore, one *R. blasii* and two

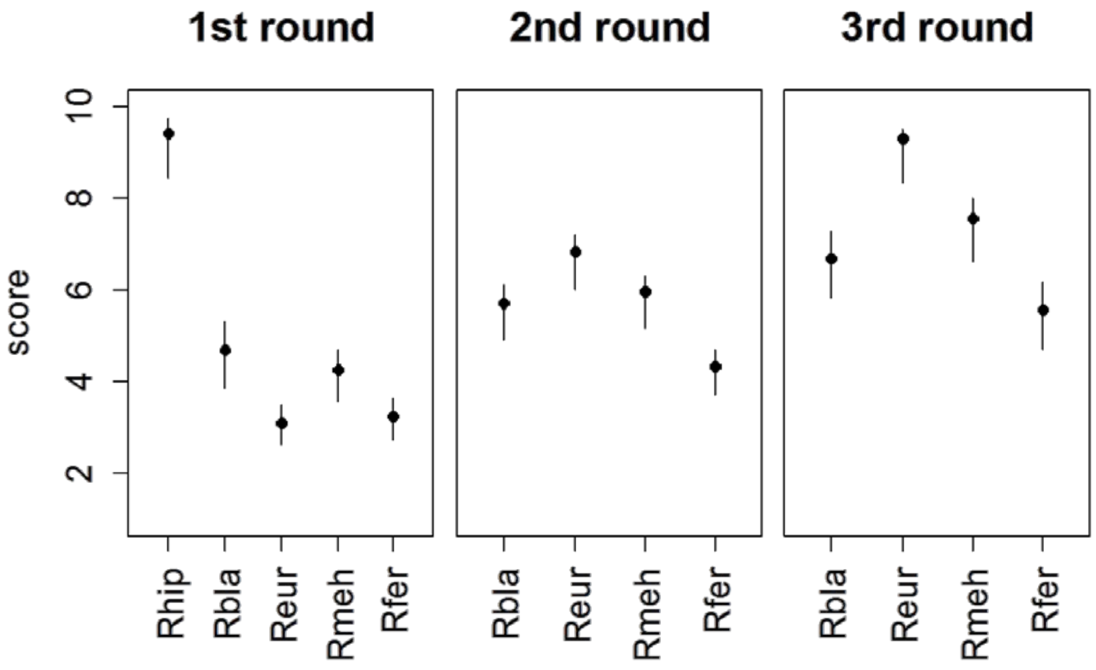

Figure 4.3: Model prediction of performance score per round and species for an obstacle spacing of 25 cm. Rhip = *R. hipposideros*, Rbla = *R. blasii*, Reur = *R. euryale*, Rmeh = *R. mehelyi*, Rfer = *R. ferrumequinum*. The predictions were from a clmm model with the lowest AIC; the lines show the bootstrapped 95% confidence intervals. In some cases, the model predictions are not in the centre of the confidence intervals because of the influence of extreme values. For example, the score of *R. euryale* in the 3rd round was high (9 or 10) in all cases, with the exception of one case, where a medium score of 5 was reached and a low score (1-3) was not given.

Table 4.2: Median values of length, area measurements and mass with 95 % confidence intervals calculated with bootstrapping method.

	R. hipposideros (N = 2)	*R. blasii* (N = 7)	*R. euryale* (N = 6)	*R. mehelyi* (N = 6)	*R. ferrumequinum* (N = 6)	*p*	*p* (intermediate)
Mass [g]	5.6 (5.5 - 5.7)	11.15 (10.7 - 11.5)	11.7 (11.3 - 12.0)	14.2 (13.4 - 15.7)	18.8 (17.8 - 20.0)	$p = 0.001$	$p = 0.017$
Wing span [cm]	24.5 (24.3 - 24.7)	30.0 (29.5 - 30.0)	30.6 (30.2 - 31.2)	33.2 (32.8 - 33.4)	38.0 (36.2 - 38.6)	$p = 0.001$	$p = 0.008$
Wing area [cm^2]	109.7 (109.7 - 109.8)	153.6 (148.0 - 157.0)	164.6 (160.2 - 168.9)	179.4 (169.5 - 181.3)	231.4 (215.1 - 241.7)	$p < 0.001$	$p = 0.006$
Hand-wing length [cm]	5.8 (5.7 - 6.0)	6.8 (6.6 - 6.9)	7.4 (7.2 - 7.5)	7.8 (7.6 - 8.0)	8.9 (8.3 - 9.1)	$p < 0.001$	$p = 0.005$
Hand-wing area [cm^2]	18.8 (18.3 - 19.3)	24.6 (24.4 - 25.0)	27.4 (26.3 - 28.18)	30.2 (28.5 - 31.0)	37.4 (34.5 - 40.0)	$p < 0.001$	$p = 0.007$
Arm-wing length [cm]	4.7 (4.6 - 4.8)	5.5 (5.4 - 5.6)	5.7 (5.7 - 5.9)	6.1 (5.9 - 6.2)	6.7 (6.5 - 7.2)	$p = 0.001$	$p = 0.010$
Arm-wing area [cm^2]	26.4 (25.6 - 27.2)	37.0 (35.7 - 38.7)	39.9 (38.2 - 41.1)	43.9 (39.9 - 45.6)	58.0 (51.7 - 60.1)	$p = 0.003$	$p = 0.102$ (ns)
Digit 5 [cm]	5.1 (5.0 - 5.2)	5.7 (5.6 - 5.7)	6.0 (5.7 - 6.2)	6.3 (6.1 - 6.8)	7.0 (6.8 - 7.3)	$p = 0.003$	$p = 0.016$
Tail length [cm]	2.5 (2.4 - 2.6)	2.5 (2.3 - 2.7)	2.8 (2.5 - 2.9)	2.8 (2.5 - 2.9)	3.8 (3.5 - 4.0)	$p = 0.049$	$p = 1.0$ (ns)
Tail area [cm^2]	8.4 (8.43 - 8.44)	10.3 (9.1 - 10.7)	10.1 (9.0 - 11.8)	12.6 (10.5 - 13.4)	16.1 (15.4 - 18.1)	$p = 0.008$	$p = 0.635$ (ns)
Body length [cm]	4	5.7 (5.5 - 5.8)	5.5 (5.5 - 5.7)	5.9 (5.7 - 6.0)	6.6 (6.4 - 6.8)	$p = 0.006$	$p = 0.134$ (ns)
Upper arm & body width [cm]	6.0 (5.9 - 6.2)	7.4 (7.3 - 7.5)	7.7 (7.5 - 8.0)	7.9 (7.8 - 8.1)	9.2 (9.0 - 9.6)	$p = 0.002$	$p = 0.040$
Body area [cm^2]	12.5 (12.0 - 12.9)	21.5 (19.7 - 22.1)	20.1 (19.6 - 21.5)	21.7 (20.2 - 22.0)	27.9 (26.7 - 30.1)	$p = 0.023$	$p = 1.0$ (ns)

N refers to the number of individuals and ns refers to not significant. The values of the five species or only the three intermediate species were compared with a Kruskal-Wallis test (df = 4 for all five species and df = 2 for the intermediate species) and the resulting *p*-values were corrected for multiple comparisons with Bonferroni correction factor. Significance is indicated by the corrected *p*-values.

Table 4.3: Medians of ratios and indices with 95 % confidence intervals.

	R. hipposideros (N = 2)	*R. blasii* (N = 7)	*R. euryale* (N = 6)	*R. mehelyi* (N = 6)	*R. ferrumequinum* (N = 6)	*p*	*p* (intermediate)
Aspect ratio	5.47 (5.39 - 5.55)	5.88 (5.74 - 5.92)	5.69 (5.55 - 5.91)	6.12 (6.09 - 6.33)	6.17 (5.99 - 6.34)	$p = 0.005$	$p = 0.016$
Relative wing loading [N/m^2]	28.18 (27.9 - 28.5)	31.25 (30.95 - 33.45)	30.57 (29.43 - 31.96)	33.11 (31.46 - 34.52)	30.28 (28.24 - 32.53)	$p = 0.219$ (ns)	$p = 0.426$ (ns)
Tip length ratio	1.24 (1.21 - 1.27)	1.22 (1.21 - 1.27)	1.28 (1.23 - 1.32)	1.29 (1.24 - 1.34)	1.27 (1.26 - 1.34)	$p = 1.0$ (ns)	$p = 1.0$ (ns)
Tip area ratio	0.71 (0.67 - 0.75)	0.67 (0.64 - 0.69)	0.69 (0.67 - 0.71)	0.68 (0.65 - 0.76)	0.65 (0.62 - 0.71)	$p = 1.0$ (ns)	$p = 1.0$ (ns)
Wing-tip shape index	1.36 (1.29 - 1.42)	1.18 (1.09 - 1.33)	1.15 (1.06 - 1.30)	1.14 (1.04 -1.36)	1.07 (0.96 - 1.13)	$p = 0.880$ (ns)	$p = 1.0$ (ns)
Tail-to-body length ratio	0.63 (0.60 - 0.65)	0.44 (0.42 - 0.47)	0.49 (0.46 - 0.53)	0.46 (0.44 - 0.49)	0.58 (0.55 - 0.59)	$p = 0.007$	$p = 0.275$ (ns)
Tail-to-wing area ratio	0.082 (0.082 - 0.082)	0.074 (0.064 - 0.075)	0.066 (0.059 - 0.075)	0.075 (0.066 - 0.080)	0.077 (0.074 - 0.082)	$p = 0.167$ (ns)	$p = 1.0$ (ns)

N refers to the number of individuals and ns refers to not significant. The values of the five species or only the three intermediate species were compared with a Kruskal-Wallis test (df = 4 for all five species and df = 2 for the intermediate species) and the resulting *p*-values were corrected for multiple comparisons with Bonferroni correction factor. Significance is indicated by the corrected *p*-values.

R. mehelyi were observed to land on the ground in front of the obstacle course before flying through it (intentional landing).

Differences in manoeuvrability

The best model of performance score (Table S 4.1, model 19) included the independent variables spacing (effect between obstacle spacing and performance score, parameter estimate (Est.) ± standard error (SE) = 8.00 ± 0.22, z = 36.53, p < 0.001), species (see values in Table 4.4), round (Est. ± SE = 0.62 ± 0.11, z = 5.63, p < 0.001) and an interaction between the species and the round (see values in Table 4.4). The variable trial (within a spacing) did not contribute to any of the four best models (Table S 4.1). At the narrow obstacle spacings 20 cm, 25 cm and 30 cm the difference in the performance of the five species was highest (Fig. S 4.2). Furthermore, the performance of each species improved over the course of the three rounds (see as example model predictions at a spacing of 25 cm in Fig. 4.3). We reran the best model with different reference species to evaluate the difference in performance among species (Table 4.4 A). The performance of the smallest and largest species

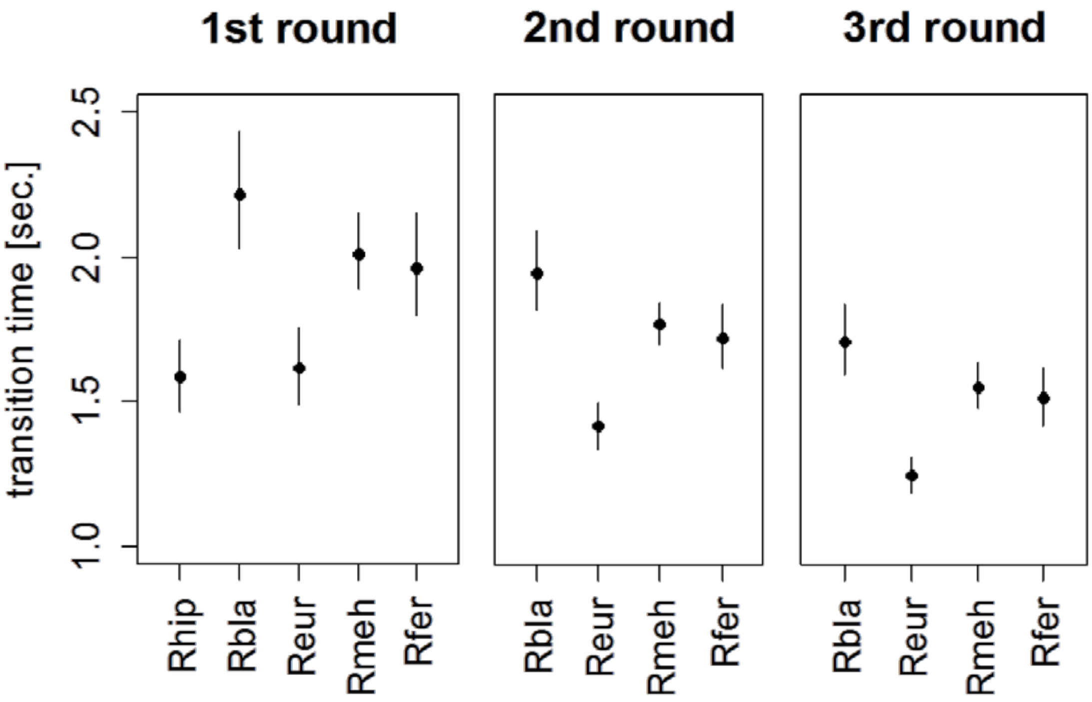

Figure 4.4: Model prediction of transition time (only for scores 6 - 10) per round and species for an obstacle spacing of 25 cm. Rhip = *R. hipposideros*, Rbla = *R. blasii*, Reur = *R. euryale*, Rmeh = R. *mehelyi*, Rfer = *R. ferrumequinum*. The predictions were from a lmer model with the lowest AICc; the lines show the bootstrapped 95 % confidence intervals.

Table 4.4: Results for the differing species within the best model with dependent variable performance score and independent variables spacing, species and round.

A)

reference species →	*R. blasii*				*R. euryale*				*R. mehelyi*				*R. ferrumequinum*			
	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*
R. hipposideros	3.29	0.60	5.46	**<0.001**	4.21	0.59	7.12	**<0.001**	3.52	0.59	5.99	**<0.001**	4.09	0.59	6.95	**<0.001**
R. blasii	-	-	-	-	-0.59	0.35	-1.68	0.093	-0.13	0.35	-0.36	0.716	0.69	0.35	1.99	**0.047**
R. euryale	0.59	0.35	1.65	0.098	-	-	-	-	0.46	0.33	1.39	0.166	1.27	0.33	3.82	**<0.001**
R. mehelyi	0.13	0.35	0.36	0.721	-0.46	0.33	-1.38	0.167	-	-	-	-	0.81	0.33	2.46	**0.014**
R. ferrumequinum	-0.69	0.35	-1.95	**0.051**	-1.27	0.33	-3.80	**<0.001**	-0.81	0.33	-2.46	**0.014**	-	-	-	-

B)

reference species →	*R. blasii*				*R. euryale*				*R. mehelyi*				*R. ferrumequinum*			
	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*
R. blasii : round	-	-	-	-	-1.50	0.18	-8.46	**<0.001**	-0.35	0.17	-2.11	**0.035**	-0.12	0.17	-0.71	0.481
R. euryale : round	1.50	0.18	8.46	**<0.001**	-	-	-	-	1.15	0.17	6.95	**<0.001**	1.39	0.17	8.35	**<0.001**
R. mehelyi : round	0.35	0.17	2.11	**0.035**	-1.39	0.17	-8.34	**<0.001**	-	-	-	-	0.24	0.16	1.52	0.130
R. ferrumequinum : round	0.12	0.17	0.70	0.481	-1.15	0.17	-6.95	**<0.001**	-0.24	0.16	-1.52	**0.130**	-	-	-	-

C)

reference species →	*R. blasii*				*R. euryale*				*R. mehelyi*				*R. ferrumequinum*			
	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*
R. blasii	-	-	-	-	-1.82	0.48	-3.77	**<0.001**	-0.09	0.46	-0.19	0.847	0.78	0.46	1.69	0.091
R. euryale	1.81	0.48	3.77	**<0.001**	-	-	-	-	1.73	0.46	3.77	**<0.001**	2.60	0.47	5.57	**<0.001**
R. mehelyi	0.09	0.46	0.19	0.847	-1.73	0.46	-3.77	**<0.001**	-	-	-	-	0.87	0.44	1.98	**0.048**
R. ferrumequinum	-0.78	0.46	-1.69	0.092	-2.60	0.47	-5.57	**<0.001**	-0.87	0.44	-1.98	**0.048**	-	-	-	-

In (A) values (Est. = parameter estimate, SE = standard error, z = z-value, p = p-value) are given for all five species and in (B) the values are shown for the interaction of species with round with the full dataset. C shows the values of the species for the reduced dataset with the last round only.

(*R. hipposideros* and *R. ferrumequinum*) was significantly different from each other and from the intermediate species (*R. blasii*, *R. euryale* and *R. mehelyi*), although the performance difference for *R. ferrumequinum* when compared with the second smallest species *R. blasii* was with a p-value around $p = 0.05$ only barely significant (Table 4.4 A). We did not find difference in performance score among the intermediate species. Regarding the interaction between species and round, we found a highly significant interaction for *R. euryale* and to a lesser extent for *R. mehelyi* (Table 4.4 B, Fig. 4.3).

Because performance improved across rounds and we were interested in the maximum manoeuvrability performance, we also tested for differences in performance in the last round only (no data for *R. hipposideros*). With this reduced dataset, we ran our model (without the variable round and the interaction of species to round) several times with different reference species (Table 4.4 C). There was a significant effect of species on performance score, and all pair-wise comparisons were significant except for *R. mehelyi* vs. *R. blasii,* and *R. ferrumequinum* vs. *R. blasii*.

Differences in transition time

We obtained transition times for 45 trials from the two *R. hipposideros*, 396 trials from the five *R. blasii*, 519 trials from the six *R. euryale*, 530 trials from the six *R. mehelyi* and 449 trials from the six *R. ferrumequinum* in total (including only trials with scores of 6 - 10). The average transition time at the 25-cm spacing was 1697 ms, and at the 35-cm spacing, the average transition time was reduced to 1319 ms.

The best model with the dependent variable transition time included the independent variables spacing (effect between obstacle spacing and transition time (Est. ± SE = -0.80 ± 0.08, $t = -10.46$), round (Est. ± SE = -0.13± 0.02, $t = -7.49$) and trial (Est. ± SE = -0.01 ± 0.00, $t = 3.43$, $p < 0.001$). Furthermore, the best model included an interaction between obstacle spacing and species (Est. ± SE = -0.26 - 1.13 ± 0.09 – 0.17, $t = -2.34 – 6.66$, $p < 0.001$) and an interaction between obstacle spacing and round (Est. ± SE = 0.21 ± 0.04, $t = 4.90$, $p < 0.001$). The p-values were obtained with ANOVA comparisons of the next simpler models, missing another variable in question each time. No *p-values* could be obtained for round, spacing and species alone. The bats needed less time for crossing the obstacle course when the spacing was wider (Fig. S 4.3). For the achievable narrower spacings, *R. hipposideros* and *R. euryale* had the shortest transition times, and *R. blasii* the longest. The variation in transition time for the narrow spacings 20 cm and 25 cm was highest within and among individuals (Fig S 4.3).

Relationship between morphology and flight performance – with single morphometric variables

When we tested all of the morphometric variables obtained for the five horseshoe bat species to determine their influence on the performance score in the same basic model, we found two models with a similarly low AICc value and significant *p*-values. The first model included the morphometric variable mass, and the second model included body length (Table 4.5 A and S 4.3). The third best model included the body area ($p < 0.001$).

For testing only the intermediate species, we used the data of the species *R. blasii*, *R. euryale* and *R. mehelyi* from all trials in the last round. The three models with the lowest AICc values for the intermediate horseshoe bat species included the variable mass and body length that were also found in two of the three best models of the dataset that included all five species. However, the variable body length was no longer the most important variable. Instead, the relative wing loading was found in the best model and had the only significant corrected *p*-value with $p = 0.010$ (Table 4.5 B and Table S 4.4 A).

Table 4.5: Three best basic generalized linear mixed effects models (GLMM) with response variable score and one added morphological variable (in bold) for (A) all five horseshoe bat species and (B) the last round with only the intermediate horseshoe bat species.

A)

Model	AICc	Variable	Estimate	SE	z	p
2	4923.12	Spacing	7.55	0.21	35.79	< 0.001
		Round	1.00	0.07	15.27	< 0.001
		Mass	**-0.79**	**0.14**	**-5.60**	**< 0.001**
3	4924.37	Spacing	7.58	0.21	35.80	< 0.001
		Round	0.95	0.06	14.93	< 0.001
		Body length	**-0.69**	**0.11**	**-6.21**	**< 0.001**
8	4928.43	Spacing	7.58	0.21	35.78	< 0.001
		Round	0.94	0.06	14.85	< 0.001
		Body area	**-0.71**	**0.13**	**-5.61**	**< 0.001**

B)

Model	AICc	Variable	Estimate	SE	z	p
16	947.95	Spacing	8.41	0.52	16.25	< 0.001
		Relative wing loading	**-0.71**	**0.20**	**-3.48**	**0.010**
2	951.82	Spacing	8.44	0.52	16.22	< 0.001
		Mass	**-0.59**	**0.24**	**-2.53**	0.232
3	952.30	Spacing	8.47	0.52	16.19	< 0.001
		Body length	**-0.59**	**0.25**	**-2.39**	0.338

AICc is the small-sample-size corrected version of the Akaike information criterion (AIC). The *p*-values of the morphological variables were corrected for multiple testing with a Bonferroni correction factor.

Relationship between morphology and flight performance – with multiple morphometric variables

The model selection procedure, which included several morphometric variables per model, resulted in one MAM for all five horseshoe bat species and one MAM for the intermediate species. The model including all species consisted of the morphological variables body length, relative wing loading, aspect ratio and tail-to-wing-area ratio (Table 4.6 A). For all variables, except the tail-to-wing area ratio, a significant *p*-value < 0.003 was found.

The MAM for the intermediate species consisted of the following significant morphological variables: tip length ratio, aspect ratio, body length and digit 5 length (Table 4.6 B).

Table 4.6: Model selection results shows best models with response variable score for (A) the full dataset with all five horseshoe bat species and (B) the dataset for the last round with only the three intermediate horseshoe bat species.

A)

AICc	Variable	Estimate	SE	z	*p*
4911.5	Spacing	7.55	0.21	35.75	**< 0.001**
	Round	1.02	0.07	15.12	**< 0.001**
	Body length	-0.92	0.13	-7.24	**< 0.001**
	Relative wing loading	-0.36	0.11	-3.22	**0.001**
	Aspect ratio	0.46	0.15	3.14	**0.002**
	Tail-to-wing area ratio	0.16	0.11	1.49	0.138

B)

AICc	Variable	Estimate	SE	z	*p*
943.75	Spacing	8.48	0.52	16.20	**< 0.001**
	Tip length ratio	0.77	0.19	4.16	**< 0.001**
	Aspect ratio	-0.76	0.25	-3.06	**0.002**
	Body length	-0.42	0.21	-1.97	**0.049**
	Digit 5	0.35	0.18	1.97	**0.049**

DISCUSSION

Wing morphology

Besides the overall size differences in external morphology among the five horseshoe bat species, we found several morphometric variables which had values that were not proportional to the other measures. Among the intermediate-sized species differences in overall size exist, however no significant differences were found for the arm-wing area, tail length, tail area, body length and body area, indicating that these species are not just scaled versions of the same model (not the same silhouette) and differences in shape within those body regions with non-significant differences exist.

The results for the ratios and indices support the indicated shape differences in the way that the tail length in relation to the body length (tail-to-body length ratio) when comparing all five species was significantly different. Furthermore significantly different values were found for the wing region in regard to the aspect ratio for all five species and also the intermediate species alone.

Differences in manoeuvrability and transition time

The five horseshoe bat species differed in their ability to negotiate obstacles. We found that for all species except *R. blasii*, the smaller species performed better than the larger species. Although *R. blasii* is one of the smaller species, the maximum manoeuvrability performance of the slightly larger *R. euryale* was better which indicates that overall size differences alone are not the only factors influencing performance differences.

Manoeuvrability performance increased across the three rounds, which we attribute to spatial learning (Barchi et al., 2013). In the wild, horseshoe bats consistently forage in familiar localities (Dietz et al., 2009, Motte and Libois 2002, Aihartza et al., 2003, Goiti et al., 2006) providing an opportunity to fine-tune flight paths in cluttered, vegetatively complex habitats. Improvement in performance across rounds was most distinct for *R. euryale* and to a lesser extent for *R. mehelyi.* Although we attribute performance differences among species to morphological differences, behavioural flexibility and echolocation signal design might also play role. For example, *R. blasii* has proven behavioural flexibility in prey-capture strategies (Siemers & Ivanova 2004) and performed in our experiments better than *R. euryale, R. mehelyi* and *R. ferrumequinum* on the first round of presentations, but this distinction was lost in the 2nd and 3rd rounds. We are confident, that manoeuvrability performance in the third round is the maximal performance for each species within our experiments and therefore a suitable performance for comparison among tested species and with morphology.

Differences in transition times among species were significant but small. The most manoeuvrable species, *R. hipposideros* and *R. euryale* had the lowest transition times, suggesting that greater manoeuvrability enabled faster flight through the obstacle course.

Relationship between morphology and flight performance

In the single-variable morphometric models, size played an important role in manoeuvrability performance of all five species and, to a lesser extent, of the three intermediate-size species. Body mass was the variable found in the best single-variable morphometric model for all species, reflecting the known association between mass and manoeuvrability; bats with higher mass are less manoeuvrable (Aldridge & Brigham 1988; Norberg 1994; Swartz *et al.* 2012; Voigt & Holderied 2012). Body length and body area were found in the second and third-best models. The importance of body length for manoeuvrability performance was reported in a similar study on mouse-eared bats (*Myotis myotis* and *M. blythii*) (Schmieder et al., 2014). The authors suggested that in contrast to the adjustability of the wing, body length is less flexible and may restrict tight, quick turns close to obstacles. As body length and body area relate to the same region, similar conclusions can be anticipated for the influence of body area on manoeuvrability. Our results showed that the tested *R. blasii* had a similarly long or even longer body length than the slightly larger *R. euryale*. We therefore propose that relative to size, the longer body length in *R. blasii* played a role in its poorer manoeuvrability performance.

Body mass and body length were also selected in the models of performance in the three intermediate-sized species. However, the best model consisted of the size-independent variable relative wing loading. Low wing loading is commonly associated with good manoeuvrability (Norberg & Rayner 1987). Although we did not find significant differences in relative wing loading for the intermediate (or all five) horseshoe bat species the values of all tested species are quite low compared to other species and typical for species with short and broad wings (Norberg 1994). We can therefore consider the relative wing loading as a measure of general importance for the manoeuvrability performance of the tested horseshoe bats.

In contrast to our predictions we did not find any morphometric variables related to wing size (length, area) or shape in our single-variable morphometric models. This result is in line with the results of Schmieder et al. (2014) and indicates that differences in wing morphology of closely-related bat species do not necessarily play the main role for differences in manoeuvrability performance. Unlike the single-variable models, body mass and body area did not contribute to the best multiple-variable (MAM) models. However body length remained in the best MAM models and seems therefore the most important contributor. The MAMs also highlighted the relevance of some wing parameters and ratios (tip length ratio, aspect ratio and digit 5 length). In particular, tip length ratio was selected by both the 5- and 3-species models. However, the relative wing loading (connected with mass) was found in the MAM for all five species as well. The tail-to-wing area ratio and the tip length ratio were also included in the MAM for all five species. The differences between the tested species for these two variables were not significant and for the tip length ratio compared with other species in the medium range (Norberg & Rayner 1987). We therefore consider the tail-to-wing area and the tip length ratio within a medium range as being of general importance for good manoeuvrability performance within the species.

Contrary to a former study on manoeuvrability performance in mouse-eared bats (Schmieder, Zsebők & Siemers 2014), where the tail-to-body length ratio but not the tail-to-wing area ratio was found in the MAM, in our study, the tail area clearly seems to be more important and a more reliable measure; in these horseshoe bat species, we were able to fully extend the tail membrane area with more accuracy than was possible in the study on the mouse-eared bats. A longer tail and a larger tail membrane can increase manoeuvrability and agility (Norberg 1994; Dudley 2002; Gardiner et al., 2011a; Gardiner et al. 2011b; Adams et al., 2012; Schmieder et al., 2014). The most manoeuvrable species (*R. hipposideros*) had the longest tail and largest tail area relative to size. Though, the second most manoeuvrable species (*R. euryale*) had the smallest relative tail area, which weakens the importance of the tail membrane for manoeuvrable flight performance in these species.

In the MAM model for the three intermediate species, the remaining two variables in the model were associated with wing shape, namely aspect ratio and digit 5. Manoeuvrability is associated with a low value for aspect ratio and high value for digit 5 – both indicating that the wing is relatively short and broad (Norberg & Rayner 1987; Norberg 1994; Voigt *et al.* 2010).

Interestingly, as in the single-variable models, we could not find any effect of the wing tip shape (wing-tip shape index) in the MAMs.

Conclusions

We can conclude that regarding manoeuvrability, the smallest species, *R. hipposideros,* and the middle-sized species, *R. euryale,* are best adapted to forage within dense vegetation. The species *R. blasii*, *R. mehelyi* and *R. ferrumequinum* seem less well adapted to forage in dense vegetation. Differences in overall size seem to be therefore the main mechanism providing the potential for an ecological separation of the studied species within shared habitats. The shape of the wing does not play an essential role for the manoeuvrability performance, but differences in other influencing external morphometric variables deviating from the overall size trend - especially the body length - were found and seem to play an important role.

The results show that these bats are well adapted to different microhabitats in the habitat where they occur sympatrically. However, the differences found between the five species are small, and compared with other species, all are very well adapted to forage within vegetation. In a similar experiment with greater and lesser mouse-eared bats (Schmieder et al., 2014), both species performed worse than all five horseshoe bat species.

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SUPPLEMENTARY MATERIAL

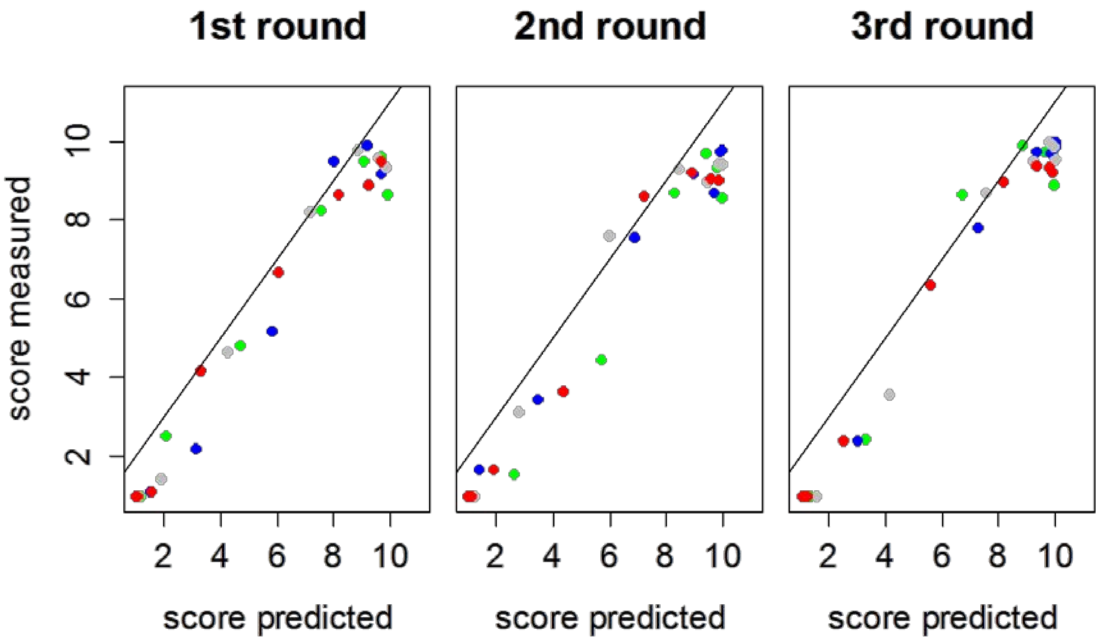

Figure S 4.1: Model fit (per round) of the lmer-model with the lowest AICc (mod19) with performance score. The coloured points represent the predicted and measured scores for the different species (green = *R. blasii*, blue = *R. euryale*, grey = *R. mehelyi* and red = *R. ferrumequinum*).

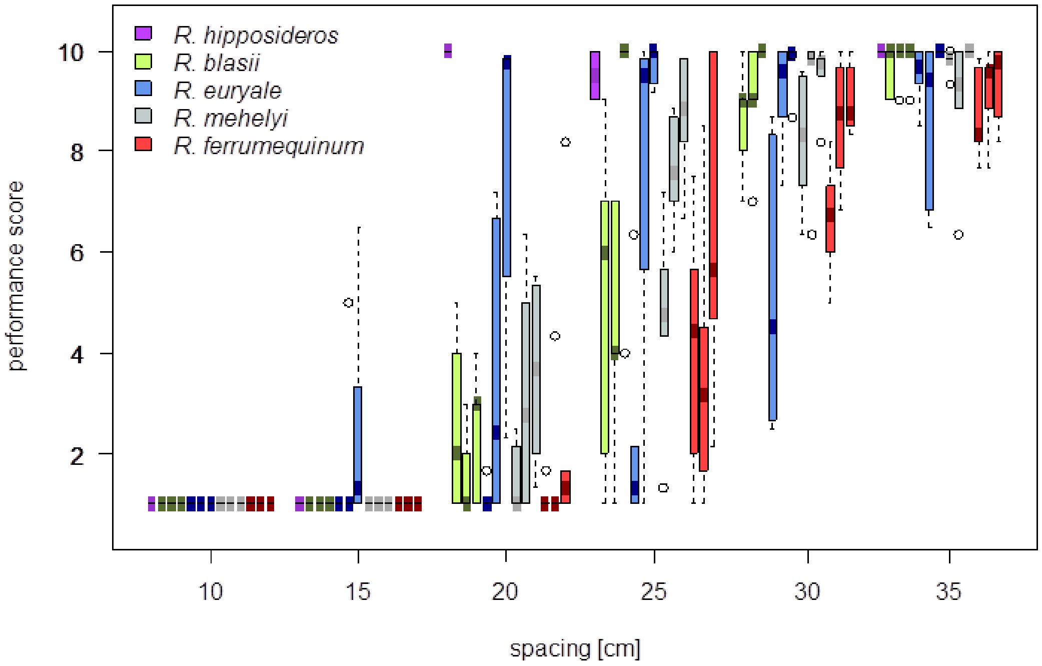

Figure S 4.2: Performance score for each tested spacing, round and species (calculated from individual averages). Bars of the same colour at a specific spacing represent rounds 1-3 from left to right. The bars represent the interquartile ranges, therein are the medians shown with horizontal black bars; the whiskers below and above represent the minimum and maximum values; the circles represent outliers.

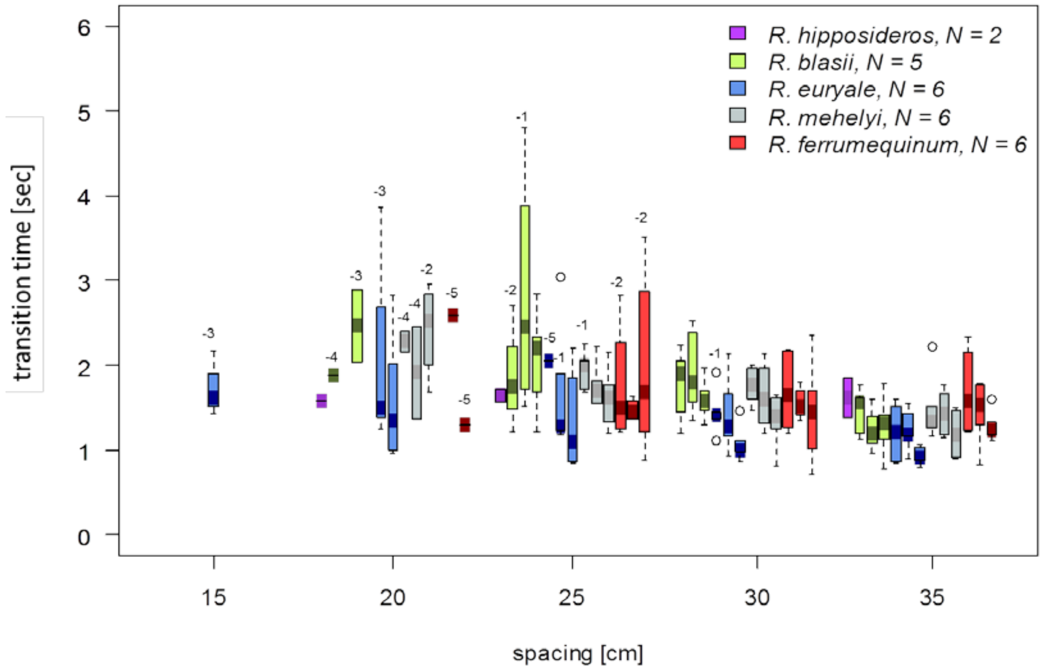

Figure S 4.3: Transition time (only for scores 6 - 10) for each spacing, round and species (calculated by individual averages). Bars with same colouration at a specific spacing represent the rounds 1-3 from left to right. The horizontal darker coloured bars are the medians; the coloured boxes represent the interquartile ranges; the whiskers below and above represent the minimum and maximum values; and the circles represent outliers. At 15-cm spacing, only one boxplot is shown for *R. euryale* in the third round; in all other cases, no transition time was available. At 20-cm spacing, transition times for *R. blasii* in the 2nd round, *R. euryale* in the 1st round and *R. ferrumequinum* in the 1st round are missing. The negative numbers above the boxplots indicate cases where transition times of individuals were missing, e.g., minus three means three individuals less**.**

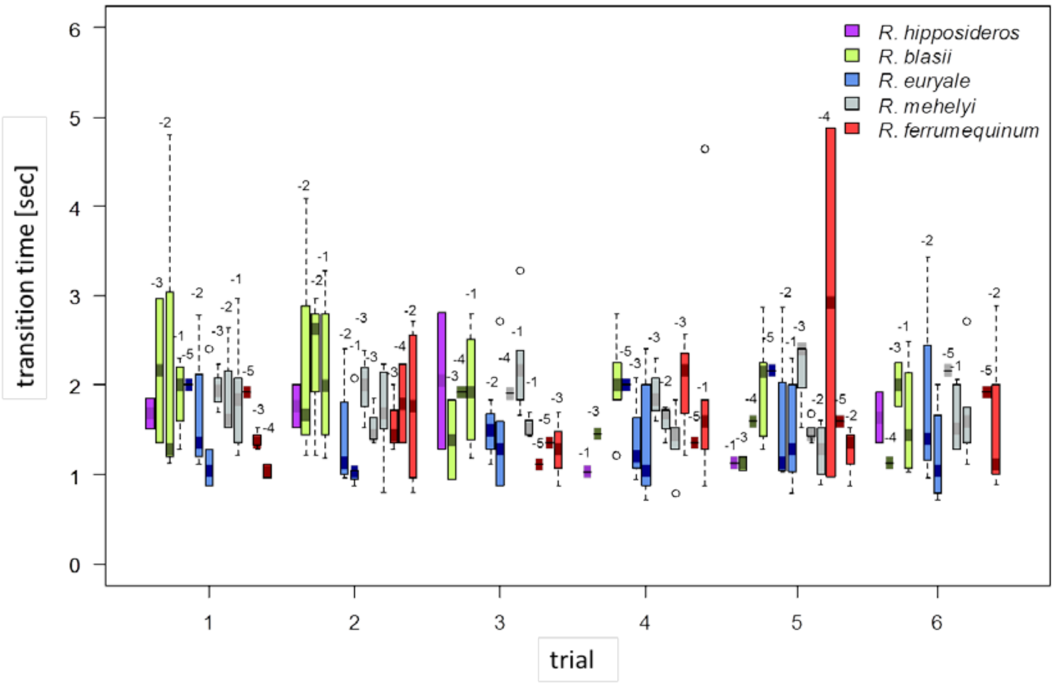

Figure S 4.4: Transition time (only for scores 6 -10) per trial, round and species (calculated by individual averages) at an obstacle distance of 25 cm. Bars with same colouration at a specific spacing represent the rounds 1-3 from left to right. The horizontal darker coloured bars are the medians; the coloured boxes represent the interquartile ranges; the whiskers below and above represent the minimum and maximum values; and the circles represent outliers. At trials 2, 3 and 6, no transition time was available for *R. euryale* in the first round, as well as for *R. ferrumequinum* in trial 6. For *R. blasii* no transition time was available at trial 4 in the second round. The negative numbers above the boxplots indicate cases where transition times of individuals were missing, e.g., minus three means three individuals less.

Table S 4.1: Finding the best model with the score as the dependent variable.

Model	AICc	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5
19	**5065.62**	**Spacing**	**Species**	**Round**	**Species:round**	
18	5066.68	Spacing	Species	Round	Spacing:species	Species:round
6	5157.40	Spacing	Species	Round		
17	5159.19	Spacing	Species	Round	Spacing:species	
9	5159.25	Spacing	Species	Round	Trial	
4	5186.76	Spacing	Round			
8	5188.63	Spacing	Round	Trial		
3	5415.00	Spacing	Species			
7	5416.62	Spacing	Species	Trial		
2	5434.69	Spacing				
5	5436.37	Spacing	Trial			
11	8718.55	Species	Round			
13	8720.55	Species	Round	Trial		
14	8731.63	Round				
15	8733.64	Round	Trial			
10	8784.28	Species				
12	8786.25	Species	Trial			
1	8791.50	1				
16	8793.47	Trial				

The same background colour (grey or white) indicates models that are not at least two units different in regard to the AICc value. Best model indicated in bold type.

Table S 4.2: Finding the best model with the transition time as the dependent variable.

Model	AICc	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5	Variable 6	Variable 7
27	**944.08**	**Spacing**	**Species**	**Round**	**Trial**	**Spacing:species**	**Spacing:round**	
26	961.47	Spacing	Species	Round	Trial	Spacing:species		
17	983.69	Spacing	Round	Trial	Spacing:round			
20	984.26	Spacing	Species	Round	Spacing:round			
8	987.08	Spacing	Round	Trial				
4	987.77	Spacing	Round					
19	988.09	Spacing	Round	Trial	Spacing:round	Round:trial		
18	990.22	Spacing	Round	Trial	Round:trial			
21	996.35	Spacing	Species	Round	Trial	Species:round		
9	1000.08	Spacing	Species	Round	Trial			
6	1000.84	Spacing	Species	Round				
24	1003.26	Spacing	Species	Round	Trial	Round:trial		
5	1017.95	Spacing	Trial					
2	1019.58	Spacing						
7	1029.55	Spacing	Species	Trial				
25	1031.01	Spacing	Species	Round	Trial	Species:trial		
3	1031.24	Spacing	Species					
22	1047.43	Spacing	Species	Round	Trial	Spacing:species	Species:round	Round:trial
23	1056.09	Spacing	Species	Round	Trial	Species:round	Round:trial	
16	1407.71	Trial						
15	1410.24	Round	Trial					
1	1410.62	1						
14	1412.75	Round						
12	1416.68	Species	Trial					
10	1419.63	Species						
13	1420.34	Species	Round	Trial				
11	1422.93	Species	Round					

The same background colour (grey or white) indicates models that are not at least two units different in regard to the AICc value. Best model indicated in bold type.

Table S 4.3

A) Finding best model with each time only one morphometric variable.

Model	AICc	Variable 1	Variable 2	Variable 3	*p*-value
2	**4923.12**	**Spacing**	**Round**	**Mass**	**<0.001**
3	**4924.37**	**Spacing**	**Round**	**Body length**	**<0.001**
8	4928.43	Spacing	Round	Body area	**<0.001**
5	4932.20	Spacing	Round	Arm-wing area	**<0.001**
11	4932.22	Spacing	Round	Upper arm & body width length	**<0.001**
12	4933.52	Spacing	Round	Arm-wing length	**<0.001**
14	4933.52	Spacing	Round	Wing area	**<0.001**
7	4933.66	Spacing	Round	Wing span	**<0.001**
16	4937.21	Spacing	Round	Relative wing loading	**0.006**
10	4937.90	Spacing	Round	Hand-wing area	**0.001**
13	4938.30	Spacing	Round	Hand-wing length	**0.002**
6	4941.78	Spacing	Round	Digit 5	**0.019**
9	4942.10	Spacing	Round	Tail area	**0.020**
19	4943.12	Spacing	Round	Wing-tip shape index	**0.044**
4	4945.03	Spacing	Round	Tail length	0.147
18	4945.60	Spacing	Round	Tip area ratio	0.224
15	4946.63	Spacing	Round	Aspect ratio	0.514
1	4949.35	Spacing	Round		
20	4950.23	Spacing	Round	Tail-to-body length ratio	1.0
21	4950.49	Spacing	Round	Tail-to-wing area ratio	1.0
17	4951.34	Spacing	Round	Tip length ratio	1.0

The *p*-values of the morphological variables were corrected for multiple testing with a Bonferroni correction factor.

B) Model selection process to find better model with several morphometric variables.

Final selection A	Final selection B	Final selection C
Mass	**Body length**	**Body area**
Wing-tip shape index	Wing-tip shape index	Wing-tip shape index
Tail-to-body length ratio	Relative wing loading	Relative wing loading
Tail-to-wing area ratio	Aspect ratio	Aspect ratio
Tip length ratio	Tail-to-body length ratio	Tail-to-wing area ratio
	Tail-to-wing area ratio	Tail-to-body length ratio
	Tip length ratio	Tip length ratio
Best model A:	**Best model B:**	**Best model C:**
Mass	Body length	Relative wing loading
Tail-to-body length ratio	Aspect ratio	Body area
Tail-to-wing area ratio	Relative wing loading	
Tip length ratio	Tail-to-wing area ratio	
AICc	AICc	AICc
4915.63	4911.52	4919.3

We made three final selections with not correlating variables each one consisting of one of the variables from the three best models with only one morphometric variable.

Table S 4.4:

A) Finding best model for data of intermediate species only with each time one morphometric variable.

Model	AICc	Variable 1	Variable 2	*p*-value
16	947.95	Spacing	**Relative wing loading**	**0.010**
2	951.82	Spacing	**Mass**	0.232
3	952.30	Spacing	**Body length**	0.338
15	953.51	Spacing	Aspect ratio	0.810
8	953.65	Spacing	Body area	0.918
1	955.28	Spacing		
17	955.48	Spacing	Tip length ratio	1.0
20	955.82	Spacing	Tail-to-body length ratio	1.0
7	956.15	Spacing	Wing span	1.0
21	956.58	Spacing	Tail-to-wing area ratio	1.0
9	956.70	Spacing	Tail area	1.0
18	956.79	Spacing	Tip area ratio	1.0
5	957.02	Spacing	Arm-wing area	1.0
13	957.13	Spacing	Hand-wing length	1.0
12	957.17	Spacing	Arm-wing length	1.0
14	957.22	Spacing	Wing area	1.0
4	957.28	Spacing	Tail length	1.0
11	957.31	Spacing	Upper arm & body width length	1.0
6	957.32	Spacing	Digit 5	1.0
10	957.33	Spacing	Hand-wing area	1.0
19	957.33	Spacing	Wingtip shape index	1.0

The *p*-values of the morphological variables were corrected for multiple testing with a Bonferroni correction factor.

B) Model selection process to find best model with several morphometric variables.

Final selection A	Final selection B	Final selection C
Relative wing loading	**Mass**	**Body length**
Body length	Body area	Aspect ratio
Body area	Tip length ratio	Body area
Tip length ratio	Tail-to-body length ratio	Tip length ratio
Tail-to-body length ratio	Tail-to-wing area ratio	Tail-to-body length ratio
Wing span	Tip area ratio	Tail-to-wing area ratio
Tail-to-wing area ratio		Tip area ratio
Tip area ratio		Arm-wing area
		D5
Best model A:	**Best model B:**	**Best model C:**
Body length	Mass	Body length
Tip length ratio	Tip length ratio	Aspect ratio
Tail-to-body length ratio		Tip length ratio
Tail-to-wing area ratio		D5
AICc	AICc	AICc
947.05	950.33	943.75

We made three final selections with not correlating variables each one consisting of one of the variables from the three best models with only one morphometric variable.

Table S 4.5: Correlation matrix of morphometric measures and ratios from all five horseshoe bat species

	Mass	**Body length**	**Tail length**	**Digit 5**	**Upper arm & body width length**	**Wing span**	**Arm-wing length**	**Hand-wing length**	**Wing area**	**Arm-wing area**	**Hand-wing area**	**Tail area**	**Body area**	**Aspect ratio**	**Relative wing loading**	**Tip length ratio**	**Tip area ratio**	**Wing-tip shape index**	**Tail-to-bodylength ratio**	**Tail-to-wingarea ratio**
Mass	1.00	0.92	0.81	0.89	0.92	0.96	0.90	0.91	0.94	0.93	0.93	0.86	0.90	0.72	0.31	0.32	-0.24	-0.48	0.17	0.11
Body length	0.92	1.00	0.71	0.81	0.91	0.93	0.87	0.87	0.90	0.87	0.88	0.80	0.92	0.69	0.31	0.25	-0.27	-0.48	-0.05	0.00
Tail length	0.81	0.71	1.00	0.73	0.80	0.80	0.78	0.81	0.85	0.83	0.86	0.85	0.74	0.43	-0.11	0.31	-0.12	-0.33	0.66	0.30
Digit 5	0.89	0.81	0.73	1.00	0.92	0.93	0.93	0.92	0.92	0.91	0.91	0.85	0.86	0.66	0.04	0.25	-0.23	-0.44	0.18	0.12
Upper arm & body width length	0.92	0.91	0.80	0.92	1.00	0.96	0.97	0.93	0.98	0.96	0.93	0.89	0.95	0.58	0.04	0.16	-0.34	-0.50	0.16	0.09
Wing span	0.96	0.93	0.80	0.93	0.96	1.00	0.96	0.97	0.98	0.96	0.97	0.89	0.92	0.73	0.14	0.33	-0.22	-0.47	0.14	0.09
Arm-wing length	0.90	0.87	0.78	0.93	0.97	0.96	1.00	0.95	0.97	0.96	0.94	0.89	0.91	0.57	-0.03	0.14	-0.32	-0.47	0.16	0.10
Hand-wing length	0.91	0.87	0.81	0.92	0.93	0.97	0.95	1.00	0.96	0.93	0.98	0.86	0.85	0.68	0.06	0.45	-0.13	-0.45	0.21	0.07
Wing area	0.94	0.90	0.85	0.92	0.98	0.98	0.97	0.96	1.00	0.99	0.97	0.92	0.94	0.59	0.00	0.25	-0.30	-0.51	0.24	0.13
Arm-wing area	0.93	0.87	0.83	0.91	0.96	0.96	0.96	0.93	0.99	1.00	0.94	0.92	0.93	0.53	0.00	0.19	-0.41	-0.60	0.24	0.16
Hand-wing area	0.93	0.88	0.86	0.91	0.93	0.97	0.94	0.98	0.97	0.94	1.00	0.88	0.86	0.66	0.05	0.39	-0.10	-0.37	0.28	0.09
Tail area	0.86	0.80	0.85	0.85	0.89	0.89	0.89	0.86	0.92	0.92	0.88	1.00	0.83	0.52	-0.11	0.17	-0.35	-0.51	0.36	0.50
Body area	0.90	0.92	0.74	0.86	0.95	0.92	0.91	0.85	0.94	0.93	0.86	0.83	1.00	0.55	0.12	0.11	-0.45	-0.59	0.06	0.01
Aspect ratio	0.72	0.69	0.43	0.66	0.58	0.73	0.57	0.68	0.59	0.53	0.66	0.52	0.55	1.00	0.51	0.53	0.17	-0.15	-0.11	0.01
Relative wing loading	0.31	0.31	-0.11	0.04	0.04	0.14	-0.03	0.06	0.00	0.00	0.05	-0.11	0.12	0.51	1.00	0.26	0.12	-0.01	-0.46	-0.28
Tip length ratio	0.32	0.25	0.31	0.25	0.16	0.33	0.14	0.45	0.25	0.19	0.39	0.17	0.11	0.53	0.26	1.00	0.50	-0.08	0.18	-0.07
Tip area ratio	-0.24	-0.27	-0.12	-0.23	-0.34	-0.22	-0.32	-0.13	-0.30	-0.41	-0.10	-0.35	-0.45	0.17	0.12	0.50	1.00	0.82	0.12	-0.20
Wing-tip shape index	-0.48	-0.48	-0.33	-0.44	-0.50	-0.47	-0.47	-0.45	-0.51	-0.60	-0.37	-0.51	-0.59	-0.15	-0.01	-0.08	0.82	1.00	0.03	-0.17
Tail-to-bodylength ratio	0.17	-0.05	0.66	0.18	0.16	0.14	0.16	0.21	0.24	0.24	0.28	0.36	0.06	-0.11	-0.46	0.18	0.12	0.03	1.00	0.46
Tail-to-wingarea ratio	0.11	0.00	0.30	0.12	0.09	0.09	0.10	0.07	0.13	0.16	0.09	0.50	0.01	0.01	-0.28	-0.07	-0.20	-0.17	0.46	1.00

All numbers in red are above a correlation factor of 0.7 and therefore considered as highly correlated. The variables marked in green belong to the best model with several morphometric variables.

Table S 4.6: Correlation matrix of morphometric measures and ratios from the intermediate species only

	Mass	Body length	Tail length	Digit 5	Upper arm & body width length	Wing span	Arm-wing length	Hand-wing length	Wing area	Arm-wing area	Hand-wing area	Tail area	Body area	Aspect ratio	Relative wing loading	Tip length ratio	Tip area ratio	Wing-tip shape index	Tail-to-bodylength ratio	Tail-to-wingarea ratio
Mass	1.00	0.77	0.41	0.62	0.66	0.88	0.63	0.70	0.76	0.70	0.74	0.62	0.30	0.74	0.65	0.36	0.18	-0.04	0.11	0.31
Body length	0.77	1.00	0.40	0.26	0.26	0.57	0.26	0.33	0.40	0.43	0.38	0.65	0.18	0.62	0.68	0.21	0.01	-0.12	0.00	0.61
Tail length	0.41	0.40	1.00	0.00	0.18	0.24	0.28	0.43	0.33	0.32	0.49	0.26	-0.34	0.00	0.26	0.36	0.29	0.10	0.92	0.13
Digit 5	0.62	0.26	0.00	1.00	0.75	0.77	0.78	0.72	0.74	0.66	0.66	0.46	0.33	0.53	0.12	0.22	0.10	-0.06	-0.11	0.12
Upper arm & body width length	0.66	0.26	0.18	0.75	1.00	0.73	0.88	0.69	0.83	0.76	0.62	0.60	0.41	0.28	0.01	0.03	-0.09	-0.14	0.09	0.23
Wing span	0.88	0.57	0.24	0.77	0.73	1.00	0.78	0.88	0.91	0.81	0.86	0.65	0.28	0.75	0.34	0.46	0.19	-0.10	0.00	0.25
Arm-wing length	0.63	0.26	0.28	0.78	0.88	0.78	1.00	0.80	0.92	0.88	0.76	0.63	0.28	0.25	-0.09	0.06	-0.07	-0.14	0.20	0.21
Hand-wing length	0.70	0.33	0.43	0.72	0.69	0.88	0.80	1.00	0.92	0.81	0.92	0.56	0.11	0.47	0.08	0.64	0.26	-0.14	0.32	0.12
Wing area	0.76	0.40	0.33	0.74	0.83	0.91	0.92	0.92	1.00	0.94	0.88	0.72	0.24	0.41	0.03	0.35	0.03	-0.21	0.19	0.28
Arm-wing area	0.70	0.43	0.32	0.66	0.76	0.81	0.88	0.81	0.94	1.00	0.74	0.75	0.33	0.29	-0.01	0.23	-0.25	-0.46	0.16	0.36
Hand-wing area	0.74	0.38	0.49	0.66	0.62	0.86	0.76	0.92	0.88	0.74	1.00	0.53	-0.10	0.50	0.16	0.57	0.47	0.15	0.36	0.10
Tail area	0.62	0.65	0.26	0.46	0.60	0.65	0.63	0.56	0.72	0.75	0.53	1.00	0.25	0.29	0.11	0.11	-0.22	-0.33	0.00	0.87
Body area	0.30	0.18	-0.34	0.33	0.41	0.28	0.28	0.11	0.24	0.33	-0.10	0.25	1.00	0.23	0.18	-0.19	-0.57	-0.53	-0.46	0.16
Aspect ratio	0.74	0.62	0.00	0.53	0.28	0.75	0.25	0.47	0.41	0.29	0.50	0.29	0.23	1.00	0.70	0.46	0.36	0.11	-0.28	0.11
Relative wing loading	0.65	0.68	0.26	0.12	0.01	0.34	-0.09	0.08	0.03	-0.01	0.16	0.11	0.18	0.70	1.00	0.26	0.28	0.18	-0.02	0.13
Tip length ratio	0.36	0.21	0.36	0.22	0.03	0.46	0.06	0.64	0.35	0.23	0.57	0.11	-0.19	0.46	0.26	1.00	0.52	-0.06	0.29	-0.08
Tip area ratio	0.18	0.01	0.29	0.10	-0.09	0.19	-0.07	0.26	0.03	-0.25	0.47	-0.22	-0.57	0.36	0.28	0.52	1.00	0.82	0.30	-0.32
Wing-tip shape index	-0.04	-0.12	0.10	-0.06	-0.14	-0.10	-0.14	-0.14	-0.21	-0.46	0.15	-0.33	-0.53	0.11	0.18	-0.06	0.82	1.00	0.16	-0.30
Tail-to-bodylength ratio	0.11	0.00	0.92	-0.11	0.09	0.00	0.20	0.32	0.19	0.16	0.36	0.00	-0.46	-0.28	-0.02	0.29	0.30	0.16	1.00	-0.13
Tail-to-wingarea ratio	0.31	0.61	0.13	0.12	0.23	0.25	0.21	0.12	0.28	0.36	0.10	0.87	0.16	0.11	0.13	-0.08	-0.32	-0.30	-0.13	1.00

All numbers in red are above a correlation factor of 0.7 and therefore considered as highly correlated. The variables marked in green belong to the best model with several morphometric variables.

5. External morphology in horseshoe bats

Bat species comparisons based on external morphology: a test of traditional versus geometric morphometric approaches

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ABSTRACT

External morphology is commonly used to identify bats as well as to investigate flight and foraging behavior, typically relying on simple length and area measures or ratios. However, geometric morphometrics are increasingly used in the biological sciences to analyze variation in shape and discriminate among species and populations. Here we compare the ability of traditional versus geometric morphometric methods in discriminating between closely related bat species, in this case European horseshoe bats (Rhinolophidae, Chiroptera) based on morphology of the wing, body and tail. In addition to comparing morphometric methods, we used geometric morphometrics to detect interspecies differences as shape changes.

Geometric morphometrics yielded improved species discrimination relative to traditional methods. The predicted shape for the variation along the between group principal components revealed that the largest differences between species lay in the extent to which the wing reaches in the direction of the head. This strong trend in interspecific shape variation is associated with size, which we interpret as an evolutionary allometry pattern.

INTRODUCTION

Studies relating bat wing morphology to flight characteristics date back to the beginning of the 20th century (Revilliod, 1916; Banks, 1930; Eisentraut, 1936; Poole, 1936). Lengths and areas of wings or their parts were typically measured to compare wing morphology among species. Wing loading, the first ratio proposed, is still used for size-independent species comparison (Poole, 1936). In following decades, further ratios, such as aspect ratio, tip length ratio, tip area ratio and the wingtip shape index were defined (Struhsaker, 1961; Findley, 1972; Norberg & Rayner, 1987). Many studies of wing morphology were conducted using these ratios to compare interspecies morphology (Aldridge & Rautenbach, 1987; Norberg & Rayner, 1987; Saunders & Barclay, 1992; Norberg, 1994; Rhodes, 1995; Salsamendi et al., 2006; Zhang et al., 2007). The methods for obtaining raw morphometric variables (e.g. wing area or arm wing area) to construct such ratios often varied among studies, making results difficult to compare (Norberg & Rayner, 1987, Findley, 1972, Rhodes, 1995, Saunders &

Barclay, 1992, Stockwell 2001, Sevcik 2003, Schmieder et al. 2014). Another problem was the way in which bats were measured. That is, early studies collected measurements on museum specimens (Findley, 1972; Norberg & Rayner, 1987), while later ones relied on wing tracings from live bats (Rhodes, 1995; Brigham et al., 1997; Bowie et al., 1999; Zhang et al., 2007). Finally, in recent years, photographs of bats with fully extended wings have been analysed with image programs (Mancina et al., 2012; Voigt 2013). For a long time, differences in total wing area and shape in relation to flight and foraging performance were emphasized and in most studies the tail was assumed to play a minor role or was not measured independently. Typically, the tail area was included in measurements of the wing area together with the body or parts of the body (Norberg & Rayner, 1987, Aldridge & Rautenbach 1987, Rhodes, 1995, Saunders & Barclay, 1992, Zhang et al., 2007, Salsamendi et al., 2006). However, Schmieder et al. (2014) used two ratios to exclusively capture tail morphology and found differences between two similar species.

Since the 80s a new set of morphometric techniques has been established: geometric morphometrics (Rohlf & Marcus, 1993; Adams et al., 2004; Adams et al., 2013). This set of techniques has gained enormous popularity and has been used across a large number of taxa and questions (for a recent review see Adams et al., 2013). These methods have become popular because they permit separation of the size and shape components of morphometric variation. The resulting variables are not redundant and these approaches allow visualization of results in terms of shape changes while retaining the geometric properties of objects throughout the analysis (Zelditch et al., 2012). Moreover, geometric morphometrics allows quantifying changes in the position of anatomical structures relative to one another, which sometimes are not captured by linear morphometric techniques. Contrasting this popularity in studies of other taxa, geometric morphometrics has had limited use in studies of external morphology in bats (but see Birch, 1997; de Camargo & de Oliveira, 2012). In the present study, we quantitatively compared the ability of traditional and geometric morphometric methods to discriminate among species of bats based on external morphology. If geometric morphometrics proves to be better in capturing differences in bats' external morphology, this method could be a helpful tool where traditional morphometrics is limited (i.e. when traditional morphometrics cannot be used to discriminate among species or when shape is itself of interest). We quantitatively compared the different methods by using classification rates in discriminant analysis and we focused on bat species that are closely related and known to be very similar in morphology. We, therefore, set out to analyse the differences in morphology of the five European horseshoe bat species (Rhinolophidae, Chiroptera). These species sometimes overlap in size and they are very similar in morphology (Norberg & Rayner, 1987, Salsamendi et al., 2006, Dietz et al., 2006a, Dietz 2007; Salsamendi et al., 2012b). The smallest European horseshoe bat is *Rhinolophus hipposideros* Bechstein, 1800 and the largest is *Rhinolophus ferrumequinum* Schreber, 1774. The other three species: *Rhinolophus blasii* Peters, 1866, *Rhinolophus euryale* Blasius, 1853 and *Rhinolophus mehelyi* Matschie, 1901 are of intermediate size and sometimes difficult to differentiate. European horseshoe bats do not only, overlap in size (especially the intermediate species). In fact, all five species are known to forage in or close to vegetation (Zahn et al., 2008, Bontadina et al., 2002, Russo et al., 2002, Goiti et al., 2008, Dietz et al., 2009, Russo et al., 2005, Salsamendi et al., 2012b, Flanders & Jones, 2009) and belong to the same foraging guild - the flutter detecting foragers (Denzinger & Schnitzler 2013). In south-eastern Europe all five species occur sympatrically (Dietz et al., 2009) and can partly overlap in diet (e.g. moths (Dietz et al., 2009, Goiti et al., 2004, Salsamendi et al., 2008, Ahmim & Moali, 2013) and hunting strategies (foraging on the wing, foraging from perches and foraging on the ground) (Dietz et al., 2009, Siemers & Ivanova, 2004, Voigt et al., 2010). The species choose similar summer roosting places like caves (in the Mediterranean regions) or roof spaces and other parts within buildings (Zahn et al., 2008, Dietz et al., 2009, Salsamendi et al., 2012b, Flanders & Jones, 2009). Although the phylogenetic relationships of this group are not fully resolved, all phylogenies published to date agree in considering *R. mehelyi* and *R. euryale* to be closely-related (Bogdanowicz & Owen, 1992; Jones et al., 2002, Guillén-Servent et al., 2003). *R. hipposideros* seems most distantly related to the other four horseshoe bat species and *R. blasii* is considered as a sister group of *R. ferrumequinum* (Stoffberg et al., 2010).

MATERIAL AND METHODS

Ethics statement

Capture and handling of bats was in accordance with recommendations of the Canadian Council on Animal Care on bats (2003) and the EUROBATS Resolution (2014) and was licensed by the responsible Bulgarian authorities (MOEWSofia and RIOSV-Ruse, field permit numbers 297/09.03.2011, 465/29.06.2012, 554/20.01.2014). The mentioned field permits authorized us to capture and measure (including taping of bats for making wing pictures) the studied species at the differing capture sites (Table S 5.1). Officials from the Bulgarian Ministry of Environment and Water (MOEW) inspected our work in accordance with Section 8, Article 23, Paragraph 3 and 4 of the Bulgarian Biodiversity Law. According to Bulgarian laws no further ethical approval by a committee is required for a non-invasive study. These procedures were not part of a routine care or monitoring project. No bats were harmed. After the experiments all bats were released in good health at their respective capture sites.

Animals

We caught bats in a harp trap (Faunatech, Victoria, Australia), mist nets (Ecotone, Sopot, Poland) or hand nets at the entrances of caves, in or near abandoned buildings in north-eastern, central and southern Bulgaria (maximal distance between capture sites approx. 260 km, Table S 5.1) between May and September in 2011, 2012 and 2014. We identified the five European horseshoe bat species using an identification key (Dietz & von Helversen, 2004). Only adult male bats with no wing injuries were used for wing pictures. We analysed pictures of 6 *Rhinolophus hipposideros*, 7 *Rhinolophus blasii*, 22 *Rhinolophus euryale*, 20 *Rhinolophus mehelyi* and 21 *Rhinolophus ferrumequinum*.

Wing pictures

Wing photographs were taken by fixing each individual with its ventral side held firmly against the board of a copy stand (custom-made, Max-Planck Institute for Ornithology, Seewiesen, Germany). The board of the copy stand was covered with graph paper and transparent self-adhesive cover film pasted on top of it. The wings and tail membrane were carefully extended (starting with the right wing, then the left wing and ending with the tail membrane) with the largest possible stretching of the wing and tail membrane and fixed each time with transparent adhesive tape (width 19 mm) to the board. For the standardization of wing position, we have considered that the upper arm was at a 90° angle to the midline of the body. Before taking pictures, we ensured that the wings and the tail were properly fixed and that no movement of the fixated body parts was possible. In the rare cases where movement of fixated body parts occurred, we readjusted and re-fixated the wing or tail before taking pictures. While fixating the bat, the head was covered with a black cotton cloth to calm the animal down and to reduce attempts of the bat to move. Each individual was fixed two times to reduce measurement error due to fixation. For each fixation, we took several digital photos with a digital camera (12 megapixel, DMC-TZ10, Panasonic, Ōsaka, Japan) mounted on the copy stand at a height of 47 cm. The tape was then carefully removed without injury. From each of the 76 individuals, we chose the three best pictures (one or two per fixation) which were then used in downstream traditional and geometric morphometric analyses. A picture was chosen as best picture if there was no blurriness in the picture, the wings were fixated symmetrically, the wing and tail membranes were fully extended and the head was straight and pointed towards the plate.

Measurements

For traditional morphometrics, we measured the right wing, tail and body to obtain multiple lengths (hand wing length, arm wing length, wing span) and areas (arm wing area, hand wing area, tail area and wing area) (Fig 5.1), using an image processing program (Adobe Photoshop, version 13.0.1, Adobe Systems, San Jose, USA). We also digitized 17 landmarks on the right side of each specimen (Fig 5.2), using tpsDig (Rohlf, 2013). From landmark coordinates, we obtained using the program TMorphGen6 of the IMP package (Sheets & Zelditch, 2003), linear distances between the landmarks 1 and 7 (3rd digit), 7 and 9 (5th digit), 5 and 6 (first phalanx of 4th digit) as well as 4 and 5 (second phalanx of 4th digit). For reduction of measurement error in geometric morphometrics, for each bat we subjectively determined and digitized the best two pictures of the best fixation and the best picture of the other fixation. We quantified the measurement error present in the dataset obtained with the above-mentioned experimental design using a Procrustes ANOVA (Klingenberg & McIntyre, 1998), which showed that measurement error was small relative to the variation among individuals and among species (Table S 5.2). The resulting coordinates were then averaged - thus further reducing measurement error (Arnqvist & Mårtensson 1998, Fruciano et al., 2012, Fruciano et al., 2011a) - for each bat after a generalized Procrustes analysis (Rohlf & Slice, 1990) in MorphoJ (Klingenberg, 2011). As a preliminary MANCOVA showed that the interaction between species and centroid size was not significant, residuals of a pooled within-group regression of shape on centroid size (accounting for 16.08% of total variance) were obtained to take into account intra-species allometry and these were used in subsequent analyses.

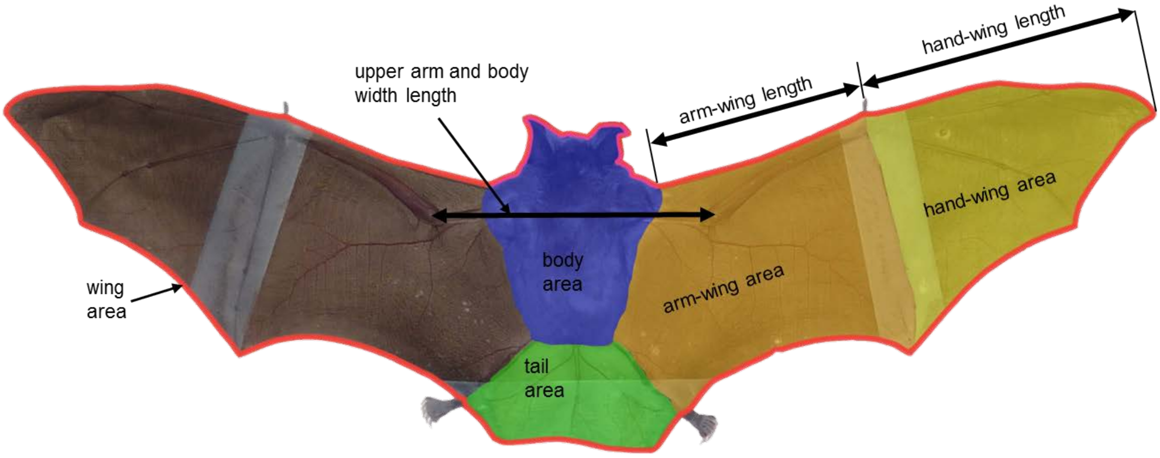

Figure 5.1. Length and area measurements taken for each analysed wing photograph. These measures were used for methods 1 and 2.

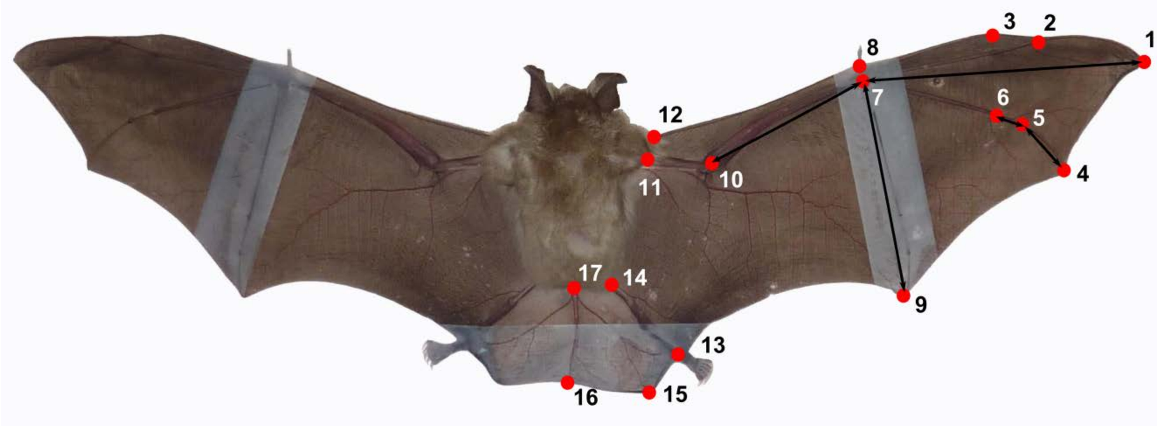

Figure 5.2. Landmarks used to generate data for methods 3 and 4. All landmarks were used in the geometric morphometric approach (method 4). Arrows show the linear distances that were taken for method 3.

Comparison of morphometric methods in species discrimination

In this study we compared 4 morphometric methods for their ability in discriminating bat species based on external morphology. The first method involved ratios and other measures that are thought to be “size-independent” (i.e. corrected for allometry): tip length ratio, tip area ratio, wing tip shape index, aspect ratio and wing loading (Norberg & Rayner, 1987) (Table S 5.3). The second method includes measures from the first method except that wing loading is replaced by relative wing loading (which is less dependent on size) and that the tail-to-wing area ratio is added to the other variables (Norberg & Rayner, 1987, Norberg 1994, Schmieder et al. 2014). In the third method we followed Dietz and colleagues (Dietz et al., 2006a), using residuals of regression on forearm length for each of the length measurements of digits 3 and 5 and also the first and second phalanges of the fourth digit. These three methods represent the traditional morphometric methods most commonly used to analyse external bat morphology of European horseshoe bats. In a fourth and final method we employed geometric morphometrics using the set of landmarks defined above. We used landmarks only on the right wings to maintain consistency with the other methods and because preliminary analyses on a subset of the specimens showed a lower measurement error due to fixation, as compared to a symmetric configuration of landmarks on both sides of the bat.

To compare methods, we used the correct classification rate of discriminant analysis estimated using a leave-one-out cross-validation procedure. We obtained discriminant analyses and correct classification rates both for pairwise comparisons among species and using a single discriminant analysis on all the species at the same time (canonical variate analysis). We obtained discriminant functions and correct classification rates for the pairwise comparisons among species using geometric morphometric data (method 4) in MorphoJ. All the other discriminant functions and correct classification rates were computed in SPSS (Version 21.0, IBM Corp. Armonk, NY). Given that linear discriminant analysis is known to have artefactually high classification rates at increasing number of dimensions (Mitteroecker & Bookstein, 2011), for the geometric morphometric dataset, we also performed discriminant analyses on, respectively, the first two, three, ten, seventeen and twenty-five principal components. The first twenty-five principal components were chosen performing in the R package nFactors (Raiche, 2010) the Anderson's test (Anderson, 1963), as suggested by Mitteroecker and Bookstein (2011) for dimensionality reduction prior to discriminant analyses. The first two, three, ten and seventeen principal components were chosen arbitrarily as lower numbers of principal components.

To test for the presence of a species signal in the raw geometric morphometric data prior to allometric correction, we also performed discriminant analyses on the geometric morphometric dataset obtained from the measurement reduction procedure without subjecting it to the regression-based removal of the allometric component.

Geometric morphometrics – testing and visualizing differences among species

In addition to the comparison of different morphometric methods, we exploited the advantages of geometric morphometrics by further analysing the geometric morphometric dataset and visualizing differences among species as shape changes. All the analyses were performed on the right-side configurations described above. However, to visualize results we reflected the configurations of points obtained as results (Fruciano et al., 2011b), thus producing more easily interpretable “bat-like” symmetric displays.

To visualize patterns of variation among species, we used between-group principal component analysis (Boulesteix, 2005). This method has been suggested to produce ordinations that are preferable to the commonly used scatterplots of canonical variate scores (Mitteroecker & Bookstein, 2011) and is increasingly used in geometric morphometric studies (Fruciano et al., 2014, Franchini et al., 2014) as the ordinations do not exaggerate the extent of separation between groups. To better interpret variation along the first between-group principal component (bwgPC1) - which was computed based on data after a pooled within-group regression on centroid size and is therefore already corrected for intra-specific allometry - in terms of evolutionary allometry, we regressed bwgPC1 scores on centroid size. We tested for pairwise differences in mean shape among species using the permutational procedure based on Procrustes distances implemented in MorphoJ (10,000 permutations).

Differences between species were visualized through wireframe graphs of each species' mean shape relative to the grand mean.

RESULTS

Comparison of morphometric methods in species discrimination

There are clear differences in correct classification rates across the four methods (Table 5.1 and Table S 5.4). The poorest classification rate was found for method 1 (Norberg & Rayner, 1987) which uses ratios related to the wing, followed by method 2 (Norberg & Rayner, 1987, Norberg 1994, Schmieder et al. 2014) which uses ratios related to wing and tail and method 3 (Dietz et al., 2006a) which employs linear measurements on the wing. The latter two methods were rather similar in correct classification rates. Method 4 (data obtained through geometric morphometrics) achieved the greatest success as correct classification was achieved with 94.7% accuracy when comparing all the species (canonical variate analysis) and ranged between 84.6% and 100% in the pairwise comparison (Table 5.1). Consistent across-methods among-species differences in correct classification were found. For instance, *R. hipposideros* showed consistently high correct classification rates in all methods. Correct classification was higher for geometric morphometrics relative to traditional methods also when the geometric morphometric dataset was subjected to dimensionality reduction (i.e. when discriminant analysis was performed on a subset of principal components; Table S 5.5). Discriminant analyses on the geometric morphometric dataset containing allometric variation produced lower correct classification rates when compared to the geometric morphometric dataset obtained after allometric correction. However, correct classification rates in the former case were still higher than the ones obtained using a traditional morphometric dataset.

In fact, the cross-validated correct classification rate for the CVA on the geometric morphometric dataset containing allometric variation was 93.4% (the same percentage of correct classification is obtained both using the full-dimensional space and using the first 25 principal components) and on average 91.63% in pairwise comparisons (range 76.92–100%). This shows that bat

Table 5.1: Cross-validated correct classification rates using traditional and geometric morphometrics. Correct classification rates for each pairwise comparison are provided in Table S3.

		Discriminant analysis using all the species						**Discriminant analyses for each pair of species**	
Data acquisition	**Method**	**Overall rate**	***R. hipposideros***	***R. blasii***	***R. euryale***	***R. mehelyi***	***R. ferrumequinum***	**Average rate**	**Range of rates**
Traditional morphometrics	1	37.0	66.7	28.6	31.8	42.1	31.6	68.6	46.2 - 89.3
	2	63.0	100	57.1	59.1	64.7	57.1	88.5	58.6 - 100
	3	67.1	83.3	85.7	72.7	60.0	57.1	88.7	69.0 - 100
Geometric morphometrics	4	94.7	100	100	86.4	95.0	100	95.3	84.6 - 100

species could be discriminated with geometric morphometrics even in the presence of significant allometry.

Geometric morphometrics – testing and visualizing differences among species

The first between-group principal component accounts for most (91.03 %) of the shape variation in the dataset. The second principal component accounted for a mere 4.26% percent of total variance. Interestingly, the different species show little overlap in the scatterplot of the first two between-group principal components (bwgPC), and variation along bwgPC1 mirrors inter-specific variation in body size as species with lower scores on bwgPC1 are larger (Fig. 5.3). This is confirmed by the regression of bwgPC1 scores (which are already corrected for intraspecific allometry) on centroid size, which is significant ($p<0.0001$) and accounts for 83% of the variation in bwgPC1 scores. Predicted shape for the variation along bwgPC1 (Fig 5.3) revealed that the largest differences between species lay in the extent to which the wing reaches in the direction of the head. Not much variation among species was present along bwgPC2, with the only exception that *R. blasii* has, on average, lower scores along this axis. Considering the low amount of variance explained by bwgPC2 and the fact that it is constructed, by definition, to be orthogonal to bwgPC1, differences along this direction are difficult to interpret and, possibly, of little biological significance. *R. mehelyi* and *R. euryale* showed the largest level of overlap in the scatterplot.

Permutation tests of difference in average shape were significant across all pairwise comparisons (Table 5.2). The lowest Procrustes distance was found between *R. mehelyi* and *R. euryale*, as suggested by their close position in the scatterplot of the scores on the first two between-group principal components.

Table 5.2: Pairwise Procustes distances among horseshoe bat species (above the diagonal) and *p*-values for the null hypothesis of equal means (below the diagonal).

	R. hipposideros	***R. blasii***	***R. euryale***	***R. mehelyi***	***R. ferrumequinum***
R. hipposideros	-	0.0656	0.0889	0.1278	0.2005
R. blasii	0.0003	-	0.0439	0.0760	0.1472
R. euryale	<.0001	<.0001	-	0.0418	0.1180
R. mehelyi	<.0001	<.0001	<.0001	-	0.0807
R. ferrumequinum	<.0001	<.0001	<.0001	<.0001	-

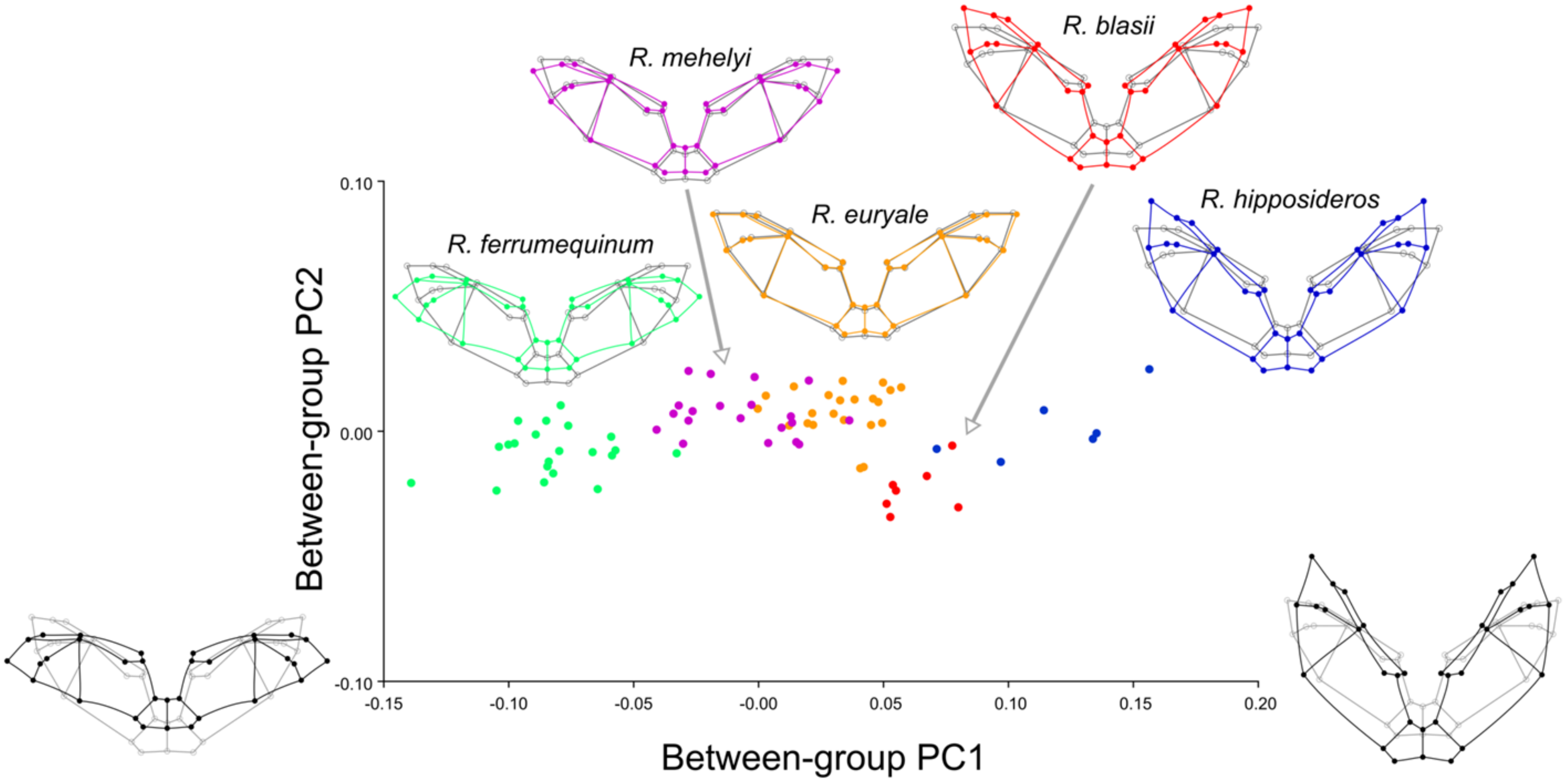

Figure 5.3: Between-group principal component analysis and average species shapes. Scatterplot of the scores along the first two between-group principal components. Overlaid, predicted shape changes along the first between-group principal component and average shape of each species. Points in the scatterplot are color-coded as the average shapes. In the plots of average species shape, the grand average shape is depicted in grey.

A narrative description of the differences of each species average shape relative to the overall average shape is provided in Table 5.3 and documents extensive variation in arm-wing, hand-wing, body and tail regions.

Table 5.3: Overview of species differences found with geometric morphometrics.

Species	Overall Comparison to Average Shape	Hand-wing Region	Arm-wing Region	Body	Tail
R. hipposideros	Wing reaches farther in cranial direction	LM 1 and 4 are farther apart resulting in LM 1 being shifted more in cranial direction hand-wing slightly longer	Arm-wing between LM 13 and 9 enlarged, between LM 8 and 9 broader	Shoulder region broader	Enlarged tail area and tail longer
R. blasii	Wing reaches farther in cranial direction	Hand-wing slightly longer	Arm-wing between LM 13 and 9 enlarged, Propatagium slightly enlarged, between LM 8 and 9 broader	Body longer	Shorter tail, enlarged (lateral direction) tail area
R. euryale	Very similar to average shape	Normal	Normal	Normal	Tail area smaller
R. mehelyi	Wing reaches less far in cranial direction	Normal	Arm-wing between LM13 and 9 shorter	Normal	Tail area smaller
R. ferrumequinum	Wing reaches less far in cranial direction	Hand-wing between LM 9 and 4 and between 4 and 1 shorter	Arm-wing between LM 13 and 9 shorter, between LM 12 and 8 slightly longer	Slightly shorter, in shoulder region broader	Tail slightly longer

DISCUSSION

We compared the ability of four multivariate approaches to discriminate between morphologically similar, closely related species of European horseshoe bats. Landmark-based geometric morphometrics performed best at species discrimination - as measured by its highest levels of correct classification in discriminant analysis. This is not surprising since geometric morphometrics has long been suggested as particularly useful in detecting even relatively small, localized changes in shape (de Camargo & de Oliveira, 2012), making this approach particularly useful in intraspecific studies (Loy, 1996). Our results suggest that geometric morphometric analyses are as useful in bats as in other groups.

This approach is not practical for rapid species identification in the field. However, it can be used to find anatomical regions where linear measures for species identification in the field should be taken, e.g. for other bat species where no field identification keys exist. Perhaps most importantly, when external morphology and species discrimination are themselves of interest geometric morphometric methods may be especially helpful.

Using geometric morphometrics, we found interspecific differences in horseshoe bats that were not detected by previously used methods. What is more interesting is that we were able to identify strong trends in interspecific shape variation associated with size. In fact, by taking into account allometric variation using a pooled within-group regression, we removed intraspecific allometric variation but not interspecific size-associated shape changes. We, therefore, conclude that the clear trend observed along the first between-group principal component - which accounts for a very high proportion of total variance - can be interpreted as a pattern of evolutionary allometry. Allometry in bats already has been described for various traits (e.g. Norberg, 1981; Hayssen & Kunz, 1996; Lupold et al., 2004; Jacobs et al., 2007; Riskin et al., 2008). The comparison of the elongated fingers of bats compared to other mammals may be the most famous example of allometry in regard to morphology (Swartz, 1997). However, to our knowledge, this is the first study to describe evolutionary allometry of external wing morphology in a detailed way and across a group of closely-related bat species.

When considering the functional implication of the shape variation we documented in horseshoe bats, we speculate that a wing reaching farther toward the head - i.e. moving in the positive direction of bwgPC1 (Fig 5.3) - might be advantageous for flight in dense vegetation. Incidents when bats touch obstacles while wings are positioned in front (ahead of the body centre) might be easier to cope with and therefore less risky. Furthermore it may be easier for bats to evaluate their ability to fly through a specifically narrow spot as well as increasing their manoeuvrability. Especially *R. hipposideros* and *R. euryale* - which have wings reaching farther towards the head - forage regularly in dense vegetation (Reiter, 2004; Russo et al., 2005; Goiti et al., 2008; Zahn et al., 2008; Dietz et al., 2009; Uhrin et al., 2012). The foraging behavior of *R. blasii* is less studied, but it is known to forage close to shrubs and hedges (Dietz et al., 2009). In contrast, the larger species *R. mehelyi* and *R. ferrumequinum* are at the negative extreme of our bwgPC1 (Fig 5.3 and Table 5.3) and these species spend more time in less-cluttered habitat foraging above or along vegetation (e.g. pastures, hedges or arable land) (Salsamendi et al., 2012b, Dietz et al., 2009, Flanders & Jones). Furthermore, both species frequently hunt from perches (flycatcher style) (Dietz 2007, Dietz et al., 2009). We assume that, for this foraging behaviour, wings reaching less far toward the head should be energetically more efficient during flight. These assumptions should be tested in a biomechanics experiment since bats flight performance cannot be predicted from wing shape alone (Riskin et al., 2008, Swartz et al., 2012). We cannot determine to which extent the foraging performance of the studied bat species in different environments might be influenced by wing shape alone as opposed to body size as we have shown that these co-vary across species. Former studies, however, have reported that smaller species have better flight performance close to or within cluttered environments (Aldridge 1985, Aldridge & Rautenbach 1987, Stockwell 2001, Schmieder et al. 2014).

An interesting possibility to test in the future is that our results may describe a more general phenomenon, i.e. bats foraging in dense vegetation have wings reaching farther towards the head compared to bats foraging in edge or open space. Norberg (1981) reported that the wings of some bat species show strong convergence with some bird wings, e.g. Mollosid bats have wings similar to the ones of swifts and swallows. Geometric morphometrics is scarcely used to study wing morphology also in birds (but see Brewer & Hertel, 2007). It is, therefore, possible that future geometric morphometric studies on birds will allow further (and more precise) tests of the parallelism across taxa of the relationship between wing shape and its functional significance.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Table S 5.1: Locality and method of capture for all individuals used in this study.

Species	ID	Year	District	Latitude	Longitude	Capture site	Method
R. hipposideros	Rhip003 - 004	2011	Ruse	43.8	25.9	Rocky niche	Handnet
R. hipposideros	Rhip001	2014	Gabrovo	42.8	25.5	Stanchov	Mist net
R. hipposideros	Rhip003, 004	2014	Lovech	42.9	24.5	Barima	Handnet
R. hipposideros	Rhip005	2014	Ruse	43.5	25.9	Ostritsa	Handnet
R. blasii	Rbla001 - 007	2011	Kardzhali	41.4	25.5	Samara	Mist net
R. euryale	Reur004 - 007	2011	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. euryale	Reur012	2011	Lovech	43.2	25.0	Mandrata	Mist net
R. euryale	Reur016 - 017	2011	Ruse	43.6	26.0	Zorovitza	Mist net
R. euryale	Reur001, 003, 004, 006, 007, 009	2012	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. euryale	Reur001, 004 - 011	2014	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. mehelyi	Rmeh001 - 008	2011	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. mehelyi	Rmeh005, 006, 008 - 011	2012	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. mehelyi	Rmeh003 - 008	2014	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. ferrumequinum	Rfer002, 003	2011	Ruse	43.7	27.0	Gabarnika	Handnet
R. ferrumequinum	Rfer004, 005	2011	Lovech	43.2	24.4	Parnitzite	Mist net
R. ferrumequinum	Rfer006, 009	2011	Gabrovo	42.4	25.1	Central Balkan	Mist net
R. ferrumequinum	Rfer001, 002, 004, 006, 008	2012	Lovech	43.2	24.4	Parnitzite	Mist net
R. ferrumequinum	Rfer001	2014	Ruse	43.5	25.9	Orlova Chuka	Harp trap
R. ferrumequinum	Rfer002 - 007	2014	Lovech	42.9	24.5	Barima	Mist net
R. ferrumequinum	Rfer008	2014	Ruse	43.6	25.9	Tabachka	Mist net
R. ferrumequinum	Rfer009 - 010	2014	Ruse	43.5	25.9	Ostritsa	Handnet

Table S 5.2. Procrustes ANOVA on repeated mesures of shape in the geometric morphometric dataset. SS=sum of squares; MS=mean squares; df=degrees of freedom.

Term	SS	MS	df	F	p	Pillai trace	p
Species	0.06669718	0.00055581	120	5.33	<.0001	3.36	<.0001
Individual	0.22215037	0.00010430	2130	2.97	<.0001	20.65	<.0001
Fixation	0.07783701	0.00003506	2220	9.38	<.0001		
Residual (picture)	0.00325134	0.00000374	870				

Table S 5.4: Cross-validated correct classification rates for each pairwise species comparison across different morphometric methods. Rates are expressed as percentages. Species abbreviations as follows: Rhip = *R. hipposideros*, Rbla = *R. blasii*, Reur = *R. euryale*, Rmeh = *R. mehelyi*, Rfer = *R. ferrumequinum*.

Data acquisition	Method	Rhip	Rbla	Reur	Rmeh	Rfer	
Traditional morphometrics	Method 1	-					**Rhip**
		46.2	-				**Rbla**
Range: 46.2 - 89.3		89.3	58.6	-			**Reur**
		88.0	76.9	61.0	-		**Rmeh**
		88.0	53.8	58.5	65.8	-	**Rfer**

Data acquisition	Method	Rhip	Rbla	Reur	Rmeh	Rfer	
Traditional morphometrics	Method 2	-					**Rhip**
		92.3	-				**Rbla**
Range: 58.6 - 100		100.0	58.6	-			**Reur**
		100.0	95.8	89.7	-		**Rmeh**
		100.0	82.1	93.0	73.7	-	**Rfer**

Data acquisition	Method	Rhip	Rbla	Reur	Rmeh	Rfer	
Traditional morphometrics	Method 3	-					**Rhip**
		76.9	-				**Rbla**
Range: 69.0 - 100		100.0	100.0	-			**Reur**
		100.0	100.0	69.0	-		**Rmeh**
		70.4	82.1	93.0	95.1	-	**Rfer**

Data acquisition	Method	Rhip	Rbla	Reur	Rmeh	Rfer	
Geometric morphometrics	Method 4	-					**Rhip**
		84.6	-				**Rbla**
Range: 84.6 - 100		100.0	100.0	-			**Reur**
		100.0	85.2	85.7	-		**Rmeh**
		100.0	100.0	100.0	97.6	-	**Rfer**

S 5.3 Table. List of all traditional morphometric variables used for the discriminant analysis and description of how the measures were taken and what general functional importance they have for flight performance.

Measure / ratio	Reference	Refers to Method	Measuring method/tool	How measured / computed?	Region on bat	Functional importance for flight performance
handwing length	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	length measure	right wing	mainly related to overall size differences: smaller species should generally perform better in confined and cluttered space and larger species should perform poorer [1,2]
armwing length	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	length measure	right wing	
handwing area	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	area measure	right wing	
armwing area	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	area measure	right wing	
wing area	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	area measure	wing, body and tail	
wing span	Norberg & Rayner 1987	Methods 1 & 2	Photoshop	length measure to body center	right wing and body	
tip length ratio	Norberg & Rayner 1987	Methods 1 & 2	Computation	ratio: handwing length / armwing length	right wing	ratios related to the wing-tip shape: species with more rounded wing-tips (higher values for wing-tip shape index) should be able to fly more slowly and to be more manoeuvrable which favours flight in cluttered habitat [1,2]
tip area ratio	Norberg & Rayner 1987	Methods 1 & 2	Computation	ratio: handwing area / armwing area	right wing	
wing tip shape index	Norberg & Rayner 1987	Methods 1 & 2	Computation	index: tip area ratio / (tip length ratio - tip area ratio)	right wing	
wing loading	Norberg & Rayner 1987	Method 1	Computation	weight*g / wing area	wing, body and tail	bats with low wing loadings can generally fly at lower speeds than bats with higher wing loadings, for species foraging close to or within vegetation a low wing loading is advantageous [1,2]
relative wing loading	Norberg 1994	Method 2	Computation	weight*g / wing area*weight^(1/3)	wing, body and tail	
aspect ratio	Norberg & Rayner 1987	Methods 1 & 2	Computation	wing span^2 / wing area	wing, body and tail	a high aspect ratio indicates narrow wings and a low aspect ratio indicates broader wings, the latter is in combination with low wing loading considered as favourable for flight within cluttered environment [1,2]
tail area	Schmieder et al. 2014	Method 2	Photoshop	photoshop area measure	tail	a larger tail membrane can increase manoeuvrability and agility and favours therefore flight in cluttered habitat [2,3]
tail-to-wing area ratio	Schmieder et al. 2014	Method 2	Computation	tail area / wing area - tail area	wing, body and tail	
forearm length	Dietz et al. 2006	Method 3	Caliper	length measure folded wing	right wing	mainly related to overall size differences: smaller species should generally perform better in confined and cluttered space and larger species should perform poorer [1,2]
Digit 3	Dietz et al. 2006	Method 3	TMorphGen6 /Matlab	distance between landmarks 1 and 7	right wing	
Digit 5	Dietz et al. 2006	Method 3	TMorphGen6 /Matlab	distance between landmarks 7 and 9	right wing	
1st phalanx of 4th finger	Dietz et al. 2006	Method 3	TMorphGen6 /Matlab	distance between landmarks 5 and 6	right wing	
2nd phalanx of 4th finger	Dietz et al. 2006	Method 3	TMorphGen6 /Matlab	distance between landmarks 4 and 5	right wing	

1. Norberg UM, Rayner JMV (1987) Ecological Morphology and Flight in Bats (Mammalia, Chiroptera) - Wing Adaptations, Flight Performance, Foraging Strategy and Echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 316: 337-419.
2. Norberg UM (1994) Wing Design, Flight Performance, and Habitat Use in Bats. In: Wainwright PC, Reilly SM, editors. Ecological Morphology, Integrative Organismal Biology. Chicago: The University of Chicago Press. pp. 205-239.
3. Schmieder DA, Zsebők S, Siemers BM (2014) The tail plays a major role in the differing manoeuvrability of two sibling species of mouse-eared bats (*Myotis myotis* and *Myotis blythii*). Canadian Journal of Zoology 92: 965-977.
4. Dietz C, Dietz I, Siemers BM (2006) Wing measurement variations in the five European horseshoe bat species (Chiroptera : Rhinolophidae). Journal of Mammalogy 87: 1241-1251.

6. General Discussion

In this thesis, I studied the morphological and mechanical adaptations of two similar, closely related and sympatrically occurring bat species with respect to artificial vegetation simulating their foraging habitat.

Despite the similarities of the tested mouse-eared bats and the horseshoe bats, it was possible to differentiate species in both ensembles in manoeuvrability performance and morphological measures.

Within both ensembles, the smaller species performed better in the obstacle courses. The small size does come with some disadvantages. The smaller sized bats have generally smaller jaws and reduced bite force (Santana et al., 2010). This is possibly why smaller bats generally feed on smaller and softer prey (Freeman and Lemen, 2007) – a relation that was also found in the studied species (Andreas et al., 2013; Ghazali and Dzeverin, 2013).

The methods for the obstacle course experiments between the two ensembles were different regarding the presented obstacle spacing, the number of repetitions, the response variables and the model type. However, for both ensembles, the best models with a response variable related to manoeuvrability performance consisted of the same important morphological variables or variables of the same morphological regions. The relevance of these morphological variables for the manoeuvrability performance in the experiments was therefore verified and might be of more general importance. The most important morphological variables for improved manoeuvrability were related to the body region (shorter body length or smaller body area), heaviness (lower mass or lower relative wing loading), tail region (longer tail length or larger tail area), the relation of hand-wing to arm-wing regions (tip length ratio and tip area ratio) and the relation of wing length to wing width (lower aspect ratio). In former studies (Norberg and Rayner, 1987; Norberg 1994) it was shown, that the slower flying and more manoeuvrable species had, in general, more rounded wing-tips.

In contrast, I could not find a main effect of the wing-tip shape relating to differences in manoeuvrability. Therefore, I anticipate that the shape of the wing-tip might be of lower importance for differences in manoeuvrability performance of closely related, similar and sympatric species.

When comparing the manoeuvrability performance of the tested mouse-eared bats with the performance of the tested horseshoe bats, all horseshoe bats performed better than the mouse-eared bats. On average, the narrowest space that the mouse-eared bats could negotiate without touching any obstacle was 43 cm for *M. blythii* and 45 cm for *M. myotis*. In the horseshoe bats, the narrowest space navigable in flight ranged from 20 cm (average for *R. euryale* and *R. hipposideros*) to 25 cm (average for *R. ferrumequinum*). The performance of the *Myotis* species may have increased slightly if the individuals were tested in more trials. Nevertheless, the overall size seemed to play a major role for these performance differences since all horseshoe bat species, except for *R. ferrumequinum,* were smaller than the two mouse-eared bat species.

In terms of overall size, *M. blythii* was very similar to *R. ferrumequinum* (e.g., similar body length and wing span). In contrast, the relative wing loading and the aspect ratio were higher in *M. blythii* compared with *R. ferrumequinum*. Although the tail length was longer and the tail area was larger for *M. blythii*, *R. ferrumequinum* performed much better in the manoeuvrability experiment. These results weaken the importance of the tail region and the body region and strengthen the importance of other measurements, such as the relative wing loading and aspect ratio, which is in line with earlier study results on distantly related bat species (Norberg and Rayner, 1987; Norberg 1994).

From the morphology alone, I was able to show that in the body region and tail region - which were neglected in former studies with wing pictures – differences can be found among similar species. The newly introduced ratios for tail-to-body length and tail-to-wing area were very helpful in finding differences with reference to the tail region. Unfortunately, no such ratio exists for the body region, and instead of defining a new ratio, size-independent differences in this region could finally be proven with the help of the geometric morphometrics method. The geometric morphometrics method was the best method to use to find differences in shape between similar species. The differences observed in the overall shape within the horseshoe bats – smaller bats having wings reaching further towards the head – have not been recorded in other studies on bats. Though, the main relevance of these differences to flight mechanics is not yet clear. The results from the obstacle course experiments with the horseshoe bat species indicate that a wing closer towards the head may well be a feature favouring better manoeuvrability. In support of this assumption, a study on the energetic costs of short manoeuvring flight showed that the smaller horseshoe bat species (*R. euryale*) had lower energy costs than the slightly larger species (*R. mehelyi*) (Voigt et al., 2010). However, in the obstacle course experiment, the species (*R. euryale)* that was second best in manoeuvrability had a body shape very similar to the average shape of all horseshoe bats species, and the second smallest species (*R. blasii*), which performed worse had wings reaching further towards the head, this contradicts the prediction.

The most interesting flight performance in this study was found for *R. blasii,* a species that performed worse in the obstacle course than expected and therefore did not follow the general trend that was observed in the other species. The longer relative body length of *R. blasii* was confirmed by geometric morphometrics and seems, therefore, to be one main factor for its poorer manoeuvrability performance. Furthermore, an enlarged propatagium was observed in this species. The propatagium or leading edge flap is generally known to be wider in horseshoe bats than in vespertilionid bats (Dietz et al., 2006b). This membrane can be adjusted, is thought to suppress leading-edge separation at higher speeds (Song et al., 2008) and seems to produce a leading edge vortex, which could have an important function for high load manoeuvres, such as sharp turns (Koekkoek et al., 2012). I assume that the propatagium plays an important role for all tested horseshoe bats regarding manoeuvrability, but because *R. blasii* performed worse, the larger propatagium did not seem to increase the manoeuvrability performance in this case and, therefore, might be a side effect of the elongated body or have another supporting function, e.g., relating to hovering or during take-offs from ground. This species seems to take prey more readily from the ground than other horseshoe bats (Siemers and Ivanova, 2004), and in my experiments, this species landed and crawled more frequently.

Another important aspect that needs to be taken into account is the comparison of the results with taxa other than bats. Generally, a low wing loading and aspect ratio are known to enhance manoeuvrability in other flying animals, such as birds or insects, and also in aircraft. In birds, similar to studies on bats, the main emphasis concerning external morphological differences has focused on differences in wing shape. Broad wings with rounded tips and low wing loading are usually associated with good manoeuvrability (Norberg, 1995; Keast, 1996). The importance of long tails for improved manoeuvrability has also been reported in several studies (Norberg, 1995; Thomas and Balmford, 1995). I am not aware of a study comparing the manoeuvrability of closely related birds involving body length or body area measurements. Nevertheless, for insects, differences in the body region (reduction of abdominal segments in Diptera and body centre of mass closer to wing base axis in Lepidoptera) were found to increase body responsiveness (Dudley, 2002).

The results of this thesis support manoeuvrability and morphology as adaptations of the different species to their foraging habitat and to the partitioning of niches, possibly to reduce interspecies competition. However, I cannot completely rule out that other factors may have influenced these differences in morphology as well. Therefore, I will discuss some of these factors in more detail.

First of all, morphological changes can also evolve through sexual dimorphism (Hedrick and Temeles, 1989). In many bat species, sexual dimorphism in size has been observed, with females often being larger than males to compensate for higher wing loading during pregnancy and pup carrying (Myers, 1978). Furthermore, in some species, the wing dimensions of female bats were greater for the same reasons (Myers, 1978; de Camargo and Oliveira, 2012). Sexual selection can cause dimorphism as found in some bird species (e.g., widowbirds), where males have strongly elongated tails to attract females (Norberg, 1995). For this dissertation, I focused on adult male bats, with one exception: an injured female mouse-eared bat that was also tested in the obstacle course. Therefore, the morphometric changes observed in the different species were not biased by differences between the sexes. Because bats are night active and vision is of lower importance, dimorphism for visual displays, such as observed in male birds with extra-long tails, is unlikely for the tested species. Furthermore, no other morphological adaptations in regard to sexual dimorphism, except for the generally larger size of the females, are known to exist for the studied species (Arlettaz et al., 1991; Dietz et al., 2006a; Dietz, 2007).

The second factor that could cause morphological changes is the avoidance of interbreeding through reproductive isolation (Schoener, 1974). Unfortunately, only little is known about the mating systems of the tested bat species (Berthier et al., 2006, Dietz et al., 2009). In the mouse-eared bats, hybridization between the two tested species is still occurring (Berthier et al., 2006). For the European horseshoe bats, no recent hybridization events were reported. Other than morphological factors favouring reproductive isolation in the studied horseshoe bats, differences among bats have been observed in echolocation parameters (Schuchmann and Siemers, 2010; Schuchmann et al., 2012; Puechmaille et al., 2014). Additionally, differences in odour might play a role in reproductive isolation (Caspers et al., 2009; Bartonicka et al., 2010), but have not yet been reported in the studied species. From these findings, I suppose that the influence of interbreeding avoidance was rather low according to the morphological differences found in my dissertation.

In the third place, a divergence in appearance to avoid habit forming predators or in response to different hunting pressure can play a role in morphological differences (Schoener, 1974; Eklov and Svanback, 2006; Fernandez-Juricic et al., 2006). Actually in birds, an effect of body mass and wing-tip pointedness was found with respect to escape responses (Fernandez-Juricic et al., 2006). In that study, birds with higher body mass and more pointed wing-tips fled longer distances and landed further away from potential predators than species with lower body mass and more rounded wings. However, the causality of the found relationship was not unambiguous (Fernandez-Juricic et al., 2006). For bats, flight performance of more heavily loaded bats was reduced (Aldridge and Brigham, 1988; MacAyeal et al., 2011), which could result in a higher chance of being captured by predators. Nevertheless, insectivorous temperate-zone bats – such as the species studied in this dissertation – were not found to show a clear tendency to avoid apparently risky situations (e.g., moonlit nights) in regard to predators, and few observations were recorded where bats showed escape behaviours from predators (Lima and O'Keefe, 2013). Therefore, there is no evidence that the development of the observed morphological differences within the tested species in this dissertation may have been influenced by predators.

Last but not least, the morphological differences in the studied species could have evolved by chance without involving competition (Brennan, 1988). For the studied mouse-eared bats (Arlettaz et al., 1997), as well as for some of the horseshoe bats, processes reducing competition between the species were identified (Salsamendi et al., 2012; Russo et al., 2014). Therefore, I argue that the importance of the observed morphological adaptations likely lies in the reduced competition among the studied species and therefore cannot be neglected.

PERSPECTIVES

Future studies will be necessary to show whether the importance of a shorter body length and a wing reaching closer toward the head can be found in other bat species that also forage close to cluttered environments.

The obstacle course and the procedure used for testing bat manoeuvrability performance led to interesting results, but we could not entirely exclude other factors influencing the mechanical performance as suggested by Dumont et al. (2009). Measurements from maximum manoeuvrability performance testing of individuals over longer periods of time and with fewer different obstacle spacings may be necessary as the horseshoe bats improved their performance over all three rounds in the obstacle experiment. Based on earlier studies on the tested species, we assumed that the echolocation behaviour would not play a limiting role in the measured manoeuvrability performance, but besides variations in the main call frequency, differences leading to better sensory performance could be found for other echolocation parameters, such as the number and duration of call groups and pulse intervals, as suggested by recent studies (Falk et al., 2014; Kothari et al., 2014). Therefore, future studies to measure and analyse the echolocation behaviour of closely related and similar species performing in an obstacle course would be interesting.

7. Record of achievement / Abgrenzung der Eigenleistung

All chapters / manuscripts were written by me with help of my co-authors.

Chapter 3: The concept for this chapter was developed by my supervisor Dr. Björn Siemers and me. All obstacle course experiments were conducted by me. The analysis was performed by me, with help from Dr. Sándor Szebök for the ensonification of obstacles and Renate Heckel who analyzed the video recordings. The statistical analysis was performed by me with help of Dr. Sándor Szebök and other experts.

Chapter 4: The concept of this chapter was mainly developed by Dr. Björn Siemers and me with helpful comments on improvements by Prof. Tigga Kingston. All obstacle course experiments and sound recordings were conducted by me. The analysis was designed and performed by me, with help from Renate Heckel who analyzed the video recordings.
The statistical analysis was performed by me with help of different experts.

Chapter 5: The concept of this chapter was mainly developed by me with help of Dr. Carmelo Fruciano. The wing pictures were taken by me with help of Ivailo Borissov or other field assistants. The analyses as well as the statistics were made by me, Dr. Carmelo Fruciano and Hugo Benitez.

8. References

Adams, D. C., Rohlf, F. J. and Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* **71**, 5-16.

Adams, D. C., Rohlf, F. J. and Slice, D. E. (2013). A field comes of age: geometric morphometrics in the 21st century. *Hystrix-Italian Journal of Mammalogy* **24**, 7-14.

Adams, R. A., Snode, E. R. and Shaw, J. B. (2012). Flapping tail membrane in bats produces potentially important thrust during horizontal takeoffs and very slow flight. *PloS one* **7**.

Ahmim, M. and Moali, A. (2013). The diet of four species of horseshoe bat (Chiroptera: Rhinolophidae) in a mountainous region of Algeria: evidence for gleaning. *Hystrix-Italian Journal of Mammalogy* **24**, 174-176.

Aihartza, J. R., Garin, I., Goiti, U., Zabala, J. and Zuberogoitia, I. (2003). Spring habitat selection by the Mediterranean Horseshoe Bat (*Rhinolophus euryale*) in the Urdaibai Biosphere Reserve (Basque country). *Mammalia* **67**, 25-32.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**, 716-723.

Aldridge, H. and Brigham, R. M. (1988). Load carrying and maneuverability in an insectivorous bat : A test of the 5 % "rule" of radio- telemetry. *Journal of Mammalogy* **69**, 379-382.

Aldridge, H. D. J. N. (1985). Manoeuvrability and ecology in British bats. *Myotis* **23-24**, 157-160.

Alridge, H. D. J. N. and Rautenbach, I. L. (1987). Morphology, Echolocation and Resource Partitioning in Insectivorous Bats. *Journal of Animal Ecology* **56**, 763-778.

Anderson TW. (1963). Asymptotic theory for principal component analysis. *Ann Math Stat.* **34**, 122–148.

Andreas, M., Reiter, A., Cepáková, E. and Uhrin, M. (2013). Body size as an important factor determining trophic niche partitioning in three syntopic rhinolophid bat species. *Biologia* **68**, 170-175.

Arlettaz, R. (1996). Feeding behaviour and foraging strategy of free-living mouse-eared bats, *Myotis myotis* and *Myotis blythii*. *Animal Behaviour* **51**, 1-11.

Arlettaz, R. (1999). Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*. *Journal of Animal Ecology* **68**, 460-471.

Arlettaz, R., Ruedi, M. and Hausser, J. (1991). Field morphological identification of *Myotis myotis* and *Myotis blythii* (Chiroptera, Vespertilionidae): A multivariate approach. *Myotis* **29**.

Arlettaz, R., Perrin, N. and Hausser, J. (1997). Trophic resource partitioning and competition between the two sibling bat species *Myotis myotis* and *Myotis blythii*. *Journal of Animal Ecology* **66**, 897-911.

Arlettaz, R., Jones, G. and Racey, P. A. (2001). Effect of acoustic clutter on prey detection by bats. *Nature* **414**, 742-745.

Arnqvist G, Mårtensson T. (1998) Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measures of shape. *Acta Zool Acad Sci H*. **44**, 73–96.

Banks, V. (1930). The relation of weight to wing area in the flight of animals. *Journal of the Malayan Branch of the Royal Asiatic Society* **8**, 334 - 360.

Barber, J. R., Razak, K. A. and Fuzessery, Z. M. (2003). Can two streams of auditory information be processed simultaneously? Evidence from the gleaning bat *Antrozous pallidus*. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **189**, 843-855.

Barchi, J. R., Knowles, J. M. and Simmons, J. A. (2013). Spatial memory and stereotypy of flight paths by big brown bats in cluttered surroundings. *Journal of Experimental Biology* **216**, 1053-1063.

Barton, K. (2014). Multi-model inference. Model selection and model averaging based on information criteria (AICc and alike). Available: http://cran.r-project.org/web/packages/MuMIn/index.html. Accessed: 12 August 2014.

Bartonicka, T., Kanuch, P., Bimova, B. and Bryja, J. (2010). Olfactory discrimination between two cryptic species of bats *Pipistrellus pipistrellus* and *P. pygmaeus*. *Folia Zoologica* **59**, 175-182.

Bates, D., Maechler, M., Bolker, B., Walker, S., Bojesen Christensen, R.H., and Singmann, H. (2013). lme4: linear mixed-effects models using Eigen and S4. Available from http://cran.r-project.org/web/packages/lme4/index.html [accessed 15 April 2013].

Berthier, P., Excoffier, L. and Ruedi, M. (2006). Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species Myotis myotis and Myotis blythii. *Proceedings of the Royal Society B-Biological Sciences* **273**, 3101-3109.

Birch, J. M. (1997). Comparing wing shape of bats: The merits of principal-components analysis and relative-warp analysis. *Journal of Mammalogy* **78**, 1187-1198.

Bogdanowicz, W. and Owen, R. D. (1992). Phylogenetic analyses of the bat family Rhinolophidae. *Zeitschrift fur Zoologische Systematik und Evolutionsforschung* **30**, 142-160.

Bontadina, F., Schofield, H. and Naef-Daenzer, B. (2002). Radio-tracking reveals that lesser horseshoe bats (*Rhinolophus hipposideros*) forage in woodland. *Journal of Zoology* **258**, 281-290.

Boulesteix, A.-L. (2005). A note on between-group PCA. *International Journal of Pure and Applied Mathematics* **19**, 359-366.

Bowie, R. C. K., Jacobs, D. S. and Taylor, P. J. (1999). Resource use by two morphologically similar insectivorous bats (*Nycteris thebaica* and *Hipposideros caffer*). *South African Journal of Zoology* **34**, 27-33.

Brennan, A. (1988). Thinking about nature: An investigation of nature, value and ecology. Athens, USA: University of Georgia Press.

Brewer ML, Hertel F. (2007). Wing morphology and flight behavior of pelecaniform seabirds. *J Morphol*. **268**, 866–877. PMID: 17638303

Brigham, R. M., Francis, R. L. and Hamdorf, S. (1997). Microhabitat use by two species of *Nyctophilus* bats: a test of ecomorphology theory. *Australian Journal of Zoology* **45**, 553-560.

Canadian Council On Animal Care (2003) CCAC species-specific recommendations on: BATS: 1–9. Available: http://www.ccac.ca/en_/standards/guidelines/additional/recommendations-species: Accessed 2014 October 21.

Canty, A. and Ripley, B. (2013). boot: Bootstrap R (S-Plus) Functions. Available from http://cran.r-project.org/web/packages/boot/index.html [accessed 17 April 2013].

Caspers, B. A., Schroeder, F. C., Franke, S., Streich, W. J. and Voigt, C. C. (2009). Odour-based species recognition in two sympatric species of sac-winged bats (*Saccopteryx bilineata*, S-leptura): combining chemical analyses, behavioural observations and odour preference tests. *Behavioral Ecology and Sociobiology* **63**, 741-749.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* **31**, 343-+.

Christensen, R. H. B. (2014). Ordinal regression models for ordinal data. Available at: http://www.cran.r-project.org/package=ordinal/.

Clarin, T. M., Ruczynski, I., Page, R. A. and Siemers, B. M. (2013). Foraging ecology predicts learning performance in insectivorous bats. *PloS one* 8, e64823.

Darwin, C. (1859). On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life London: John Murray.

Davis, R. (1968). Wing defects in a population of pallid bats. *American Midland Naturalist* **79**, 388-395.

de Camargo, N. F. and de Oliveira, H. F. M. (2012). Sexual Dimorphism in *Sturnira lilium* (Chiroptera, Phyllostomidae): Can Pregnancy and Pup Carrying Be Responsible for Differences in Wing Shape? *PloS one* **7**.

Denzinger, A. and Schnitzler, H.-U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Frontiers in physiology* **4**, 164.

Dietz, C. (2007). Aspects of ecomorphology in the five European horseshoe bats (Chiroptera: Rhinolophidae) in the area of sympatry. Dissertation: Eberhard Karls Universität Tübingen. Available: http://www.fledermaus-dietz.de/publications/publications.html. Accessed 21 October 2014.

Dietz, C. and von Helversen, O. (2004). Illustrated identification key to the bats of Europe. Available: http://www.fledermaus-dietz.de/publications/publications.html. Accessed 21 October 2014.

Dietz, C., Dietz, I. and Siemers, B. M. (2006a). Wing measurement variations in the five European horseshoe bat species (Chiroptera : Rhinolophidae). *Journal of Mammalogy* **87**, 1241-1251.

Dietz, C., von Helversen, O. and Nill, D. (2009). Bats of Britain, Europe and northwest Africa. London, U.K.: A and C Black Publishers Ltd.

Dietz, C., Dietz, I., Ivanova, T. and Siemers, B. M. (2006b). Effects of forearm bands on horseshoe bats (Chiroptera : Rhinolophidae). *Acta Chiropterologica* **8**, 523-535.

Dietz, M., Pir, J. B. and Hillen, J. (2013). Does the survival of greater horseshoe bats and Geoffroy's bats in Western Europe depend on traditional cultural landscapes? *Biodiversity and Conservation* **22**, 3007-3025.

Dudley, R. (2002). Mechanisms and Implications of Animal Flight Maneuverability. *Integrative and Comparative Biology* **42**, 135-140.

Dumont, E. R., Swartz, S. M., Kunz, T. H. and Parsons, S. (2009). Biomechanical approaches and ecological research for the study of bats. *Ecological and behavioral methods for the study of bats. 2nd edition.*, 436-458.

Eisentraut, M. (1936). A contribution on the mechanics of bat flights. *Zeitschrift fuer Wissenschaftliche Zoologie* **148**, 159-188.

Eklov, P. and Svanback, R. (2006). Predation risk influences adaptive morphological variation in fish populations. *The American Naturalist* **167**, 440-452.

Emde, G. von der, and Menne, D. (1989). Discrimination of insect wingbeat-frequencies by the bat Rhinolophus ferrumequinum. Journal of Comparative Physiology A 164, 663-671.

Emrich, M. A., Clare, E. L., Symondson, W. O. C., Koenig, S. E. and Fenton, M. B. (2014). Resource partitioning by insectivorous bats in Jamaica. *Molecular Ecology* **23**, 3648-3656.

Eurobats. (2010). Resolution 6.5: Guidelines on Ethics for Research and Field Work Pratices. Available: http://eurobats.eaudeweb.ro/en/document/resolution-65-guidelines-ethics-research-and-field-work-pratices: Accessed 2014 October 21.

Falk, B., Jakobsen, L., Surlykke, A. and Moss, C. F. (2014). Bats coordinate sonar and flight behavior as they forage in open and cluttered environments. *Journal of Experimental Biology* **217**, 4356-4364.

Fauth, J.E., Bernardo, J., Camara, M., Resetarits, W.J. Jr., Van Buskirk, J. and McCollum, S.A. (1996). Simplify the jargon of community ecology: A conceptual approach. American Naturalist **147**, 282-286.

Fernandez-Juricic, E., Blumstein, D. T., Abrica, G., Manriquez, L., Adams, L. B., Adams, R., Daneshrad, M. and Rodriguez-Prieto, I. (2006). Relationships of anti-predator escape and post-escape responses with body mass and morphology: a comparative avian study. *Evolutionary Ecology Research* **8**, 731-752.

Findley, J. S. (1972). Phenetic relationships among bats of the genus *Myotis*. *Systematic Zoo* **21**, 31-52.

Flanders, J. and Jones, G. (2009). Roost use, ranging behavior, and diet of greater horseshoe bats (*Rhinolophus ferrumequinum*) using a transitional roost. *Journal of Mammalogy* **90**, 888-896.

Freeman, P. W. and Lemen, C. A. (2007). Using scissors to quantify hardness of insects: do bats select for size or hardness? *Journal of Zoology* **271**, 469-476.

Franchini P, Fruciano C, Spreitzer ML, Jones JC, Elmer KR, Henning F et al. (2014). Genomic architecture of ecologically divergent body shape in a pair of sympatric crater lake cichlid fishes. *Mol Ecol.* **23**, 1828–1845. doi: 10.1111/mec.12590 PMID: 24237636.

Fruciano C, Tigano C, Ferrito V. (2011). Geographical and morphological variation within and between colour phases in Coris julis (L. 1758), a protogynous marine fish. *Biol J Linn Soc.* **104**, 148–162.

Fruciano, C., Tigano, C. and Ferrito, V. (2011). Traditional and geometric morphometrics detect morphological variation of lower pharyngeal jaw in *Coris julis* (Teleostei, Labridae). *Italian Journal of Zoology* **78**, 320-327.

Fruciano C, Tigano C, Ferrito V. (2012). Body shape variation and colour change during growth in a protogynous fish. *Environ Biol Fishes* **94,** 615–622.

Fruciano, C., Pappalardo, A. M., Tigano, C. and Ferrito, V. (2014). Phylogeographical relationships of Sicilian brown trout and the effects of genetic introgression on morphospace occupation. *Biological Journal of the Linnean Society* **112**, 387-398.

Galambos, R. (1942). The Avoidance of Obstacles by Flying Bats: Spallanzani's Ideas (1794) and Later Theories. *Isis* **34**, 132-140.

Gardiner, J. D., Codd, J. R. and Nudds, R. L. (2011a). An association between ear and tail morphologies of bats and their foraging style. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **89**, 90-99.

Gardiner, J. D., Dimitriadis, G., Codd, J. R. and Nudds, R. L. (2011b). A potential role for bat tail membranes in flight control. *PloS one* **6**.

Ghazali, M. and Dzeverin, I. (2013). Correlations Between Hardness of Food and Craniodal Traits in Nine Myotis Species (Chiroptera, Vespertilionidae). *Vestnik Zoologii* **47**, 67-76.

Goerlitz, H.R., Greif, S. & Siemers, B.M. (2008) Cues for acoustic detection of prey: insect rustling sounds and the influence of walking substrate. *Journal of Experimental Biology*, 211, 2799–2806.

Goiti, U., Aihartza, J. R. and Garin, I. (2004). Diet and prey selection in the Mediterranean horseshoe bat *Rhinolophus euryale* (Chiroptera, Rhinolophidae) during the pre-breeding season. *Mammalia* **68**, 397-402.

Goiti, U., Aihartza, J. R., Almenar, D., Salsamendi, E. and Garin, I. (2006). Seasonal foraging by *Rhinolophus euryale* (Rhinolophidae) in an Atlantic rural landscape in northern Iberian Peninsula. *Acta Chiropterologica* **8**, 141-155.

Goiti, U., Garin, I., Almenar, D., Salsamendi, E. and Aihartza, J. (2008). Foraging by Mediterranean horseshoe bats (*Rhinolophus euryale*) in relation to prey distribution and edge habitat. *Journal of Mammalogy* **89**, 493-502.

Goldberg, E. E. and Lande, R. (2006). Ecological and reproductive character displacement on an environmental gradient. *Evolution* **60**, 1344-1357.

Grant, P. R. (1972). Convergent and divergent character displacement. *Biological Journal of the Linnean Society* **4**, 39-68.

Grant, P. R. and Grant, B. R. (2008). How and Why Species Multiply: The Radiation of Darwin's Finches. Princeton University Press.

Griffin, D. R. and Galambos, R. (1941). The sensory basis of obstacle avoidance by flying bats. *Journal of Experimental Zoology* **86**, 481-506.

Grinnell, J. (1917). The niche-relationships of the California Thrasher. *Auk Cambridge Mass* **34**, (427-433).

Güttinger, R., Lustenberger, J., Beck, A. and Weber, U. (1998). Traditionally cultivated wetland meadows as foraging habitats of the grass-gleaning lesser mouse-eared bat (*Myotis blythii*). *Myotis* **41-49**.

Guillén-Servent A, Francis CM, Ricklefs RE. (2003). Phylogeny and biogeography of the horseshoe bats. In: Csorba G, Ujhelyi P, Thomas N, editors. Horseshoe bats of the world. Exeter: Pelagic Publishing Ltd. pp. xii–xxiv.

Hartridge, H. (1920). The avoidance of objects by bats in their flight. *Journal of Physiology-London* **54**, 54-57.

Hayssen, V. and Kunz, T. H. (1996). Allometry of litter mass in bats: Maternal size, wing morphology, and phylogeny. *Journal of Mammalogy* **77**, 476-490.

Hedrick, A. V. and Temeles, E. J. (1989). The evolution of sexual dimorhism in animals - Hypotheses and Tests. *Trends in Ecology & Evolution* **4**, 136-138.

Hurvich, C.M. & Tsai, C.-L. (1989) Regression and time series model selection in small samples. *Biometrika*, **76**, 297-307.

Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symp Quant Biol* **22**.

Iriarte-Diaz, J. and Swartz, S. M. (2008). Kinematics of slow turn maneuvering in the fruit bat Cynopterus brachyotis. *The Journal of experimental biology* **211**, 3478-3489.

Jacobs, D. S. and Barclay, R. M. R. (2009). Niche differentiation in two sympatric sibling bat species, *Scotophilus dinganii* and *Scotophilus mhlanganii*. *Journal of Mammalogy* **90**, 879-887.

Jacobs, D. S., Barclay, R. M. R. and Walker, M. H. (2007). The allometry of echolocation call frequencies of insectivorous bats: why do some species deviate from the pattern? *Oecologia* **152**, 583-594.

Jones, G. and Rayner, J. M. V. (1989). Foraging behavior and echolocation of wild horseshoe bats *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae). *Behavioral Ecology and Sociobiology***25**, 183-191.

Jones, G., Morton, M., Hughes, P. M. and Budden, R. M. (1993). Echolocation, flight morphology and foraging strategies of some West-African hipposiderid bats. *Journal of Zoology* **230**, 385-400.

Jones, K. E., Purvis, A., Maclarnon, A., Bininda-Emonds, O. R. P. and Simmons, N. B. (2002). A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biological Reviews (Cambridge)* **77**, 223-259.

Jones, P.L., Page, R.A., Hartbauer, M. & Siemers, B.M. (2011) Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species. *Behavioral Ecology and Sociobiology*, 65, 333–340.

Keast, A. (1996). Wing shape in insectivorous passerines inhabiting New Guinea and Australian rain forests and eucalypt forest eucalypt woodlands. *Auk* **113**, 94-104.

Kingston, T., Jones, G., Akbar, Z. and Kunz, T. H. (1999). Echolocation signal design in Kerivoulinae and Murininae (Chiroptera : Vespertilionidae) from Malaysia. Journal of Zoology 249, 359-374.

Kingston, T. (2001). Diversity, extinction risk and structure in an insectivorous bat community from Malaysia. In Graduate school of arts and sciences, Dissertation, pp. 1-303: Boston University.

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular ecology resources* **11**, 353-357.

Klingenberg, C. P. (2013). Cranial integration and modularity: insights into evolution and development from morphometric data. *Hystrix-Italian Journal of Mammalogy* **24**, 43-58.

Klingenberg CP, McIntyre GS. (1998). Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with procrustes methods. *Evolution*. 1363–1375.

Koekkoek, G., Muijres, F. T., Johansson, L. C., Stuiver, M., van Oudheusden, B. W. and Hedenstrom, A. (2012). Stroke plane angle controls leading edge vortex in a bat-inspired flapper. *Comptes Rendus Mécanique* **340**, 95-106.

Kornfield, I. and Smith, P. F. (2000). African cichlid fishes: Model systems for evolutionary biology. *Annu. Rev. Ecol. Syst.* **31**, 163-+.

Kothari, N. B., Wohlgemuth, M. J., Hulgard, K., Surlykke, A. and Moss, C. F. (2014). Timing matters: sonar call groups facilitate target localization in bats. *Frontiers in physiology* **5**, 168.

Krueger, F., Clare, E. L., Greif, S., Siemers, B. M., Symondson, W. O. C. and Sommer, R. S. (2014). An integrative approach to detect subtle trophic niche differentiation in the sympatric trawling bat species Myotis dasycneme and Myotis daubentonii. *Molecular Ecology* **23**, 3657-3671.

Kulzer, E., Nelson, J.E., Mckean, J.L. & Moehres, F.P. (1984) Prey-catching behaviour and echolocation in the Australian Ghost bat, *Macroderma gigas* (Microchiroptera: Megadermatidae). *Australian Mammalogy*, 7, 37–50.

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T. and Fleming, T. H. (2011). Ecosystem services provided by bats. *Annals of the New York Academy of Sciences* **1223**, 1-38.

Leibold, M. A. and McPeek, M. A. (2006). Coexistence of the niche and neutral perspectives in community ecology. *Ecology* **87**, 1399-1410.

Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., Holt, R. D., Shurin, J. B., Law, R., Tilman, D. et al. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* **7**, 601-613.

Levine, J. M. and HilleRisLambers, J. (2009). The importance of niches for the maintenance of species diversity. *Nature* **461**, 254-U130.

Lima, S. L. and O'Keefe, J. M. (2013). Do predators influence the behaviour of bats? *Biological Reviews* **88**, 626-644.

Loy, A. (1996). An Introduction to Geometric Morphometrics and Intraspecific Variation. In *Advances in Morphometrics*, vol. 284 eds. L. Marcus M. Corti A. Loy G. P. Naylor and D. Slice), pp. 271-273: Springer US.

Lupold, S., McElligott, A. G. and Hosken, D. J. (2004). Bat genitalia: allometry, variation and good genes. *Biological Journal of the Linnean Society* **83**, 497-507.

MacAyeal, L. C., Riskin, D. K., Swartz, S. M. and Breuer, K. S. (2011). Climbing flight performance and load carrying in lesser dog-faced fruit bats (*Cynopterus brachyotis*). *Journal of Experimental Biology* **214**, 786-793.

Mancina, C. A., Garcia-Rivera, L. and Miller, B. W. (2012). Wing morphology, echolocation, and resource partitioning in syntopic Cuban mormoopid bats. *Journal of Mammalogy* **93**, 1308-1317.

Mazerolle, M. J. (2014). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). URL http://CRAN.R-project.org/package=AICcmodavg. .

Meyer, A., Kocher, T. D., Basasibwaki, P. and Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550-553.

Mitteroecker, P. and Bookstein, F. (2011). Linear Discrimination, Ordination, and the Visualization of Selection Gradients in Modern Morphometrics. *Evolutionary Biology* **38**, 100-114.

Mogdans, J., Ostwald, J. and Schnitzler, H. U. (1988). The role of pinna movement for the localization of vertical and horizontal wire obstacles in the greater horseshoe bat, *Rhinolophus ferrumequinum*. *Journal of the Acoustical Society of America* **84**, 1676-1679.

Motte, G. and Libois, R. (2002). Conservation of the lesser horseshoe bat (*Rhinolophus hipposideros* Bechstein, 1800) (Mammalia : Chiroptera) in Belgium. A case study of feeding habitat requirements. *Belgian Journal of Zoology* **132**, 49-54.

Muijres, F. T., Johansson, L. C., Barfield, R., Wolf, M., Spedding, G. R. and Hedenström, A. (2008). Leading-edge vortex improves lift in slow-flying bats. *Science* **319**, 1250-1253.

Myers, P. (1978). Sexual Dimorphism in Size of Vespertilionid Bats. *The American Naturalist* **112**, 701-711.

Neuweiler, G. (1984). Foraging, Echolocation and Audition in Bats. *Naturwissenschaften* **71**, 446-455.

Norberg, U. M. (1981). Allometry of bat wings and legs and comparison with bird wings. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **292**, 359-398.

Norberg, U. M. (1994). Wing Design, Flight Performance, and Habitat Use in Bats. In *Ecological Morphology, Integrative Organismal Biology*, eds. P. C. Wainwright and S. M. Reilly), pp. 376. Chicago: The University of Chicago Press.

Norberg, U. M. (1995). How a Long Tail and Changes in Mass and Wing Shape Affect the Cost for Flight in Animals. *Functional Ecology* **9**, 48-54.

Norberg, U. M. and Rayner, J. M. V. (1987). Ecological Morphology and Flight in Bats (Mammalia, Chiroptera) - Wing Adaptations, Flight Performance, Foraging Strategy and Echolocation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **316**, 337-419.

Page, R.A. & Ryan, M.J. (2005) Flexibility in assessment of prey cues: frog-eating bats and frog calls. *Proceedings of the Royal Society B-Biological Sciences*, 272, 841–847.

Pfennig, D. W. and Pfennig, K. S. (2010). Character Displacement and the Origins of Diversity. *Am. Nat.* **176**, S26-S44.

Poole, E. L. (1936). Relative wing ratios of bats and birds. *Journal of Mammalogy* **17**, 412 - 413.

Price, P. W., Denno, R. F., Eubanks, M. D., Finke, D. L. and Kaplan, I. (2011). Insect Ecology - Behavior, Populations and Communities. Cambridge University Press.

Puechmaille, S. J., Borissov, I. M., Zsebok, S., Allegrini, B., Hizem, M., Kuenzel, S., Schuchmann, M., Teeling, E. C. and Siemers, B. M. (2014). Female mate choice can drive the evolution of high frequency echolocation in bats: A case study with *Rhinolophus mehelyi*. *PloS one* **9**.

Raiche G. (2010). nFactors: Parallel analysis and non graphical solutions to the Cattell Scree test. R package version 2.3.3. Available: http://cran.r-project.org/web/packages/nFactors/index.html. Accessed 24 October 2014.

Rasband, W.S. (1997–2012). ImageJ. National Institutes of Health, Bethesda, Md. Available from http://imagej.nih.gov/ij [accessed 13 January 2011].

RcoreTeam. (2013). R: A language and environment for statistical computing, (ed. V. R Foundation for Statistical Computing, Austria), pp. URL http://www.R-project.org/.

RcoreTeam. (2014). R: A language and environment for statistical computing, (ed. V. R Foundation for Statistical Computing, Austria), pp. URL http://www.R-project.org/.

Rainho, A., Augusto, A. M. and Palmeirim, J. M. (2010). Influence of vegetation clutter on the capacity of ground foraging bats to capture prey. *J. Appl. Ecol.* **47**, 850-858.

Reiter, G. (2004). The importance of woodland for *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae) in Austria. *Mammalia* **68**, 403-410.

Revilliod, P. A. (1916). A propos de l'adaption au vol chez les Microcheiroptères. *Verhandlungen der Naturforschenden Gesellschaft Basel* **27**, 156 - 183.

Rhodes, M. P. (1995). Wing morphology and flight behaviour of the golden-tipped bat, *Phoniscus papuensis* (Dobson) (Chiroptera: Vespertilionidae). *Australian Journal of Zoology* **43**, 657-663.

Riskin, D. K., Iriarte-Diaz, J., Middleton, K. M., Breuer, K. S. and Swartz, S. M. (2008). Allometry of Inertial Power during Flight in Pteropodid Bats. *Bat Research News* **49**, 164.

Rohlf, F. J. (2013). tpsDig. Department of Ecology and Evolution, State University of New York at Stony Brook. Available: http://life.bio.sunysb.edu/ morph/. Accessed: 21 October 2014.

Rohlf, F. J. and Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39**, 40-59.

Rohlf, F. J. and Marcus, L. F. (1993). A Revolution in morphometrics. *Trends in Ecology & Evolution* **8**, 129-132.

Russo, D. and Jones, G. (2002a). Identification of twenty-two bat species (Mammalia : Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. *Journal of Zoology* **258**, 91-103.

Russo, D., Jones, G. and Migliozzi, A. (2002b). Habitat selection by the Mediterranean horseshoe bat, Rhinolophus euryale (Chiroptera : Rhinolophidae) in a rural area of southern Italy and implications for conservation. *Biological Conservation* **107**, 71-81.

Russo, D., Almenar, D., Aihartza, J., Goiti, U., Salsamendi, E. and Garin, I. (2005). Habitat selection in sympatric *Rhinolophus mehelyi* and *R. euryale* (Mammalia : Chiroptera). *Journal of Zoology* **266**, 327-332.

Russo, D., Mucedda, M., Bello, M., Biscardi, S., Pidinchedda, E. and Jones, G. (2007). Divergent echolocation call frequencies in insular rhinolophids (Chiroptera): a case of character displacement? *Journal of Biogeography* **34**, 2129-2138.

Russo, D., Di Febbraro, M., Rebelo, H., Mucedda, M., Cistrone, L., Agnelli, P., De Pasquale, P. P., Martinoli, A., Scaravelli, D., Spilinga, C. et al. (2014).

What Story Does Geographic Separation of Insular Bats Tell? A Case Study on Sardinian Rhinolophids. *PloS one* **9**.

Salsamendi, E., Aihartza, J. R., Goiti, U., Almenar, D. and Garin, I. (2006). Echolocation calls and morphology in the Mehelyi`s (*Rhinolophus mehelyi*) and mediterranean (*R. euryale*) horseshoe bats: implications for resource partitioning. *Hystrix-Italian Journal of Mammalogy* **16**, 149 - 158.

Salsamendi, E., Garin, I., Arostegui, I., Goiti, U. and Aihartza, J. (2012a). What mechanism of niche segregation allows the coexistence of sympatric sibling rhinolophid bats? *Frontiers in Zoology* **9**.

Salsamendi, E., Garin, I., Almenar, D., Goiti, U., Napal, M. and Aihartza, J. (2008). Diet and prey selection in Mehelyi's horseshoe bat *Rhinolophus mehelyi* (Chiroptera, Rhinolophidae) in the south-western Iberian Peninsula. *Acta Chiropterologica* **10**, 279-286.

Salsamendi, E., Arostegui, I., Aihartza, J., Almenar, D., Goiti, U. and Garin, I. (2012b). Foraging ecology in Mehely's horseshoe bats: influence of habitat structure and water availability. *Acta Chiropterologica* **14**, 121-132.

Sandig, S., Schnitzler, H.-U. and Denzinger, A. (2014). Echolocation behaviour of the big brown bat (*Eptesicus fuscus*) in an obstacle avoidance task of increasing difficulty. *The Journal of experimental biology* **217**, 2876-2884.

Santana, S. E., Dumont, E. R. and Davis, J. L. (2010). Mechanics of bite force production and its relationship to diet in bats. *Functional Ecology* **24**, 776-784.

Saunders, M. B. and Barclay, R. M. R. (1992). Ecomorphology of Insectivorous Bats - a Test of Predictions Using 2 Morphologically Similar Species. *Ecology* **73**, 1335-1345.

Schipper, J. Chanson, J. S. Chiozza, F. Cox, N. A. Hoffmann, M. Katariya, V. Lamoreux, J. Rodrigues, A. S. Stuart, S. N. Temple, H. J. et al. (2008). The status of the world's land and marine mammals: diversity, threat, and knowledge. *Science* **322**, 225-230.

Schluter, D. (2001). Ecology and the origin of species. *Trends Ecol. Evol.* **16**, 372-380.

Schmieder, D. A., Zsebők, S. and Siemers, B. M. (2014). The tail plays a major role in the differing manoeuvrability of two sibling species of mouse-eared bats (*Myotis myotis* and *Myotis blythii*). *Canadian Journal of Zoology* **92**, 965-977.

Schmieder, D. A., Kingston, T., Hashim, R. and Siemers, B. M. (2012). Sensory constraints on prey detection performance in an ensemble of vespertilionid understorey rain forest bats. *Functional Ecology* **26**, 1043-1053.

Schneider, H. and Mohres, F. P. (1960). Die Ohrbewegungen der Hufeisenfledermäuse (Chiroptera, Rhinolophidae) und der Mechanismus des Bildhörens. *Zeitschrift Fur Vergleichende Physiologie* **44**, 1-40.

Schnitzler, H. U. (1968). Die Ultraschall-Ortungslaute der Hufeisen-Fledermäuse (Chiroptera-Rhinolophidae) in verschiedenen Orientierungssituationen. *Zeitschrift für Vergleichende Physiologie* **57**, 376-408.

Schnitzler, H. U. and Kalko, E. K. V. (2001). Echolocation by insect-eating bats. *Bioscience* **51**, 557-569.

Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science* **185**, 27-39.

Schuchmann, M. and Siemers, B. M. (2010). Variability in Echolocation Call Intensity in a Community of Horseshoe Bats: A Role for Resource Partitioning or Communication? *PloS one* **5**, e12842.

Schuchmann, M., Puechmaille, S. J. and Siemers, B. M. (2012). Horseshoe bats recognise the sex of conspecifics from their echolocation calls. *Acta Chiropterologica* **14**, 161-166.

Seehausen, O. (2006). African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B-Biological Sciences* **273**, 1987-1998.

Sevcik M. (2003). Does wing morphology reflect different foraging strategies in sibling bat species Plecotus auritus and P. austriacus? *Folia Zool* **52**: 121–126.

Sheets, H. D. and Zelditch, M. L. (2003). IMP: TMorphGen6- Traditional Morphometrics Variables Generation Utility. Dept. of Physics, Canisius College and Dept. of Geology, SUNY at Buffalo, Buffalo NY. Available: http://www3.canisius.edu/~sheets/morphsoft.html. Accessed: 21 October 2014.

Siemers, B. M. and Ivanova, T. (2004). Ground gleaning in horseshoe bats: comparative evidence from *Rhinolophus blasii*, *R. euryale* and *R. mehelyi*. *Behavioral Ecology and Sociobiology* **56**, 464-471.

Siemers, B. M. and Schnitzler, H. U. (2004). Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* **429**, 657-661.

Siemers, B.M. & Swift, S.M. (2006) Differences in sensory ecology contribute to resource partitioning in the bats *Myotis bechsteinii* and *Myotis nattereri* (Chiroptera: Vespertilionidae). Behavioral Ecology and Sociobi- ology, 59, 373–380.

Siemers, B. M., Greif, S., Borissov, I., Voigt-Heucke, S. L. and Voigt, C. C. (2011). Divergent trophic levels in two cryptic sibling bat species. *Oecologia* **166**, 69-78.

Sleep, D. J. H. and Brigham, R. M. (2003). An experimental test of clutter tolerance in bats. *Journal of Mammalogy* **84**, 216-224.

Song, A., Tian, X., Israeli, E., Galvao, R., Bishop, K., Swartz, S. and Breuer, K. (2008). Aeromechanics of membrane wings with implications for animal flight. *Aiaa Journal* **46**, 2096-2106.

Stockwell, E. F. (2001). Morphology and flight manoeuvrability in New World leaf-nosed bats (Chiroptera: Phyllostomidae). *Journal of Zoology* **254**, 505-514.

Stoffberg, S., Jacobs, D. S., Mackie, I. J. and Matthee, C. A. (2010). Molecular phylogenetics and historical biogeography of *Rhinolophus* bats. *Molecular Phylogenetics and Evolution* **54**, 1-9.

Struhsaker, T. (1961). Morphological factors regulating flight in bats. *Journal of Mammalogy* **42**, 152 - 159.

Sumer, S., Denzinger, A. and Schnitzler, H. U. (2009). Spatial unmasking in the echolocating Big Brown Bat, Eptesicus fuscus. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **195**, 463-472.

Suzuki, R. and Shimodaira, H. (2006). Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* **22**, 1540-1542.

Swartz, S. M. (1997). Allometric patterning in the limb skeleton of bats: Implications for the mechanics and energetics of powered flight. *Journal of morphology* **234**, 277-294.

Swartz, S. M., Iriarte-Diaz, J., Riskin, D. K. and Breuer, K. S. (2012). A bird? A plane? No, it's a bat: an introduction to the biomechanics of bat flight: Cambridge Univ Press, the Pitt Building, Trumpington St, Cambridge Cb2 1rp, Cambs, Uk.

Thomas, A. L. R. and Balmford, A. (1995). How natural selection shapes birds' tails. *The American Naturalist* **146**, 848-868.

Uhrin, M., Boldogh, S., Bücs, S., Paunovic, M., Mikova, E., Juhász, M., Csősz, I., Estok, P., Fulin, M., Gombkötő, P. et al. (2012). Revision of the occurrence of *Rhinolophus euryale* in in the Carpathian region, Central Europe. *Vespertilio* **16**, 289-328.

Voigt, C. C. (2013). Bat flight with bad wings: is flight metabolism affected by damaged wings? *The Journal of experimental biology* **216**, 1516-1521.

Voigt, C. C. and Holderied, M. W. (2012). High manoeuvring costs force narrow-winged molossid bats to forage in open space. *Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology* **182**, 415-424.

Voigt, C. and Lewanzik, D. (2012). ‘No cost of echolocation for flying bats’ revisited. *Journal of Comparative Physiology B* **182**, 831-840.

Voigt, C. C., Schuller, B. M., Greif, S. and Siemers, B. M. (2010). Perch-hunting in insectivorous Rhinolophus bats is related to high energy costs of manoeuvring flights. *Journal of Comparative Physiology B* **79** 877-883.

Walters, C. L., Freeman, R., Collen, A., Dietz, C., Fenton, M. B., Jones, G., Obrist, M. K., Puechmaille, S. J., Sattler, T., Siemers, B. M. et al. (2012). A continental-scale tool for acoustic identification of European bats. *Journal of Applied Ecology* **49**, 1064-1074.

Waters, D. A. and Vollrath, C. (2003). Echolocation performance and call structure in the megachiropteran fruit-bat Rousettus aegyptiacus. *Acta Chiropterologica* **5**, 209-219.

Weinberger, I. C., Bontadina, F. and Arlettaz, R. (2009). Translocation as a conservation tool to supplement relict bat colonies: a pioneer study with endangered horseshoe bats. *Endangered Species Research* **8**, 41-48.

Zahn, A., Holzhaider, J., Kriner, E., Maier, A. and Kayikcioglu, A. (2008). Foraging activity of *Rhinolophus hipposideros* on the island of Herrenchiemsee, Upper Bavaria. *Mammalian biology* **73**, 222-229.

Zelditch, M. L., Swiderski, D. L. and Sheets, H. D. (2012). Geometric Morphometrics for Biologists. New York and London: Elsevier Academic Press,.

Zhang, L., Liang, B., Parsons, S., Wei, L. and Zhang, S. (2007). Morphology, echolocation and foraging behaviour in two sympatric sibling species of bat (*Tylonycteris pachypus* and *Tylonycteris robustula*) (Chiroptera : Vespertilionidae). *Journal of Zoology* **271**, 344-351.

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10. List of presentations at scientific conferences (Selection)

2015: 4th International Berlin Bat Meeting (IBB): Movement Ecology of Bats, Berlin, Germany; Daniela Schmieder, Renate Heckel, Björn Siemers, Tigga Kingston, Talk: “Sympatric Horseshoe Bats Differ in Flight Performance in Confined Space”.

2015: Treffen der Deutschen Fledermausforscher (TDFF), Olpe, Germany; Daniela A. Schmieder, Hugo A. Benitez, Ivailo M. Borissov, Carmelo Fruciano, Poster: „Bat species comparisons based on external morphology: a test of traditional versus geometric morphometric approaches“.

2014: Size and Shape, Göttingen, Germany;
Daniela A. Schmieder, Sándor Zsebök, Björn Siemers, Kamran Safi, Poster: „Lesser Mouse Eared Bats Perform Better in Obstacle Course than Greater Mouse Eared Bats”.

2013: 106th Annual Meeting of the German Zoological Society, München, Germany; Daniela A. Schmieder, Sándor Zsebök, Björn Siemers, Kamran Safi, Poster: „Lesser Mouse Eared Bats Perform Better in Obstacle Course than Greater Mouse Eared Bats”.

2013: 16th International Bat Research Conference, San Jose, Costa Rica; Daniela Schmieder, Renate Heckel, Björn Siemers, Tigga Kingston, Talk: “Sympatric Horseshoe Bats Differ in Flight Performance in Confined Space”.

2011: Treffen der Deutschen Fledermausforscher (TDFF), Loccum, Germany; Daniela Schmieder, Markus Schuller, Björn M. Siemers, Poster: „ Maneuverability in the two sibling species *M. myotis* and *M. oxygnathus*“.

11. Index of abbreviations

AICc	Akaike information criterion corrected for small-sample-size
ANOVA	analysis of variance
AR	aspect ratio
AU	approximate unbiased p-values
B.M.S	Björn M. Siemers
BIC	Bayesian information criterion
BU	bootstrap probability values
bwgPC	between-group principal components
CF	constant frequency
clmm	cumulative link mixed model
cm	centimetre
cm^2	square centimetre
D.A.S	Daniela A. Schmieder
df	degrees of freedom
e.g.	exempli gratia = for example
est.	parameter estimate
et al.	et alii / et aliae = and others
fig.	figure
fig. S	supplementary figure
FM	frequency modulated
g	gramme
glmer	generalized linear mixed effects regression
GLMM	generalized linear mixed effects models
Hz	hertz
I	wing-tip shape index
i.e.	id est = that is
ID	individual
IMPRS	International Max Planck Research School
IR	infrared
kHz	kilohertz
km	kilometre
L_{aw}	arm-wing length
L_{bw}	body length
L_{fa}	forearm length
L_{hw}	hand-wing length
LM	landmark
lmer	linear mixed effects regression
L_{t}	tail length
L_{ws}	wing span
M.	*Myotis*
m	metres
max.	maximum / maximal
min.	minimum /minimal
mm	millimetres
MuMIn	Multi-model inference
N	sample size
N/m^2	newton per square metre, equal to pascal
p	*p*-value
R.	*Rhinolophus*
Rbla	*Rhinolophus blasii*
Reur	*Rhinolophus euryale*
Rfer	*Rhinolophus ferrumequinum*
Rhip	*Rhinolophus hipposideros*
RIOSV	Regional Inspectorate of the Ministry of Environment and Water
Rmeh	*Rhinolophus mehelyi*
RWL	relative wing loading
S	wing area
S_{aw}	arm-wing area
S_{b}	body area
SE	standard error
S_{hw}	hand-wing area
S_{t}	tail area
table S	supplementary table
TB_{l}	tail-to-body length ratio
T_l	tip length ratio
T_{S}	tip area ratio
TS_{S}	tail-to-wing area ratio
χ^2	Chi-Square